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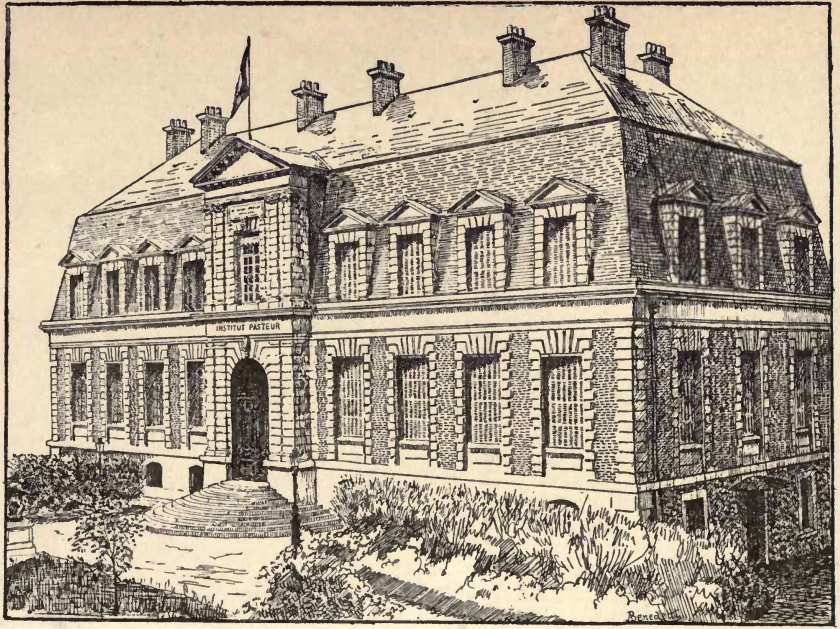
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A MANUAL OF
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PREFACE

In preparing this volume I have endeavoured to meet the requirements of those who are desirous of obtaining a knowledge of the nature and doings, for good or for evil, of those minute beings which are termed microbes or bacteria.

The mystic words 'microbes' and 'bacteria' have been hurled at the popular head with so much emphasis and so little explanation that it would not be surprising to find many people living under the misapprehension that they are minute 'fiery serpents,' which are always on the look-out for victims, and crawl about them day and night. Not a few people feel comforted by the knowledge that microbes, harmless or harmful, belong to the vegetal rather than to the animal kingdom. Such knowledge takes away the element of repulsiveness arising from the notion of microbes being internal animal parasites or entozoa.

Although microbes are minute plants, they are capable of giving rise to some of the most deadly diseases to which human flesh is heir. Consequently, a knowledge of the science of microbes, or bacteriology, is now incumbent on all medical men, sanitary engineers, chemists, physiologists, and biologists; and even intelligent householders would be all the better if they had a general knowledge of the subject detailed in the following pages.

My sincere thanks are due, and are here most gratefully tendered, to Dr. E. Klein, F.R.S.; Prof. P. F. Frankland, F.R.S.; Prof. A. Gautier (of Paris); Prof. L. Brieger (of the University of Berlin); Prof. C. Tommasi-Crudeli (of the University of Rome); Prof. I. Giglioli (of Portici, near Naples); Dr. Roux (of the Pasteur Institute); Dr. P. Miquel (of Paris); Dr. T. Lauder Brunton, F.R.S.; Mr. W. Watson Cheyne, F.R.C.S.; Dr. G. Sims Woodhead, F.R.S.E.; Dr. C. Zeiss (of Jena); and Messrs. F. E. Becker & Co. (of London) for valuable aid in various parts of the book.

In conclusion, it is hoped that this volume may contribute something towards a proper understanding and an intelligent appreciation of the important and far-reaching subject of bacteriology.

A. B. GRIFFITHS.

EDGBASTON,
January 1893.

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A MANUAL OF BACTERIOLOGY

CHAPTER I

INTRODUCTION

During the past ten years or so there is hardly a subject which has received so much attention as the Science of Bacteriology—the Study of Microbes. No one need wonder that the scientific world has been so busy in such a fruitful field of research, for it has not only been demonstrated that microbes play important parts in the processes of fermentation, putrefaction, nitrification, etc., but that many of these lowly beings are intimately connected with infectious diseases.

Phthisis, diphtheria, cholera, malaria, glanders, scarlatina, etc., have been proved to be the results of the action of certain microbes on the blood and tissues.

Infectious diseases being due to the action of certain microbes, it is necessary to isolate the microbes and to study them apart from the body. Hence the necessity of obtaining a pure culture of any particular microbe (*i.e.* its freedom from other microbes, etc.) before we can accurately study its mode of growth, multiplication, and the products it may give rise to. In fact, Dr. R. Koch[1] has laid down the following canons to ascertain whether a microbe is, directly or indirectly, the *causa causans* of a particular disease:—

(1.) The microbe in question must have been found either in the blood, lymph, or tissues of the man or animal which is suffering from, or who has died of, the disease.

(2.) The microbe taken from this medium (blood, tissues, etc.), and artifically cultivated in certain media, must be transferred from culture to culture for several successive generations, taking the precautions necessary to prevent the introduction of any other microbe into these cultures, so as to obtain the specific microbe, pure from every kind of matter proceeding from the body of the animal whence it originally came.

(3.) The microbe, thus purified by successive cultures, and reintroduced into the body of a healthy animal capable of taking the disease, ought to reproduce the disease, in the animal, with its characteristic symptoms and lesions.

(4.) Finally, it must be ascertained that the microbe in question has multiplied in the system of the animal thus inoculated, and that it exists in greater number than in the inoculating medium.

Microbes are everywhere present—in the air, in the earth, and in waters; in and on food, clothes, etc.; consequently they gain admittance into the bodies of man and animals. These microbes do not necessarily give rise to disease, for many are harmless although they may be present in the blood and tissues. Not even in the case of an infectious disease, where a certain microbe is present, can one say that it is the cause of that disease. Not until Koch's canons are fulfilled, is the experimenter justified in saying that any particular microbe is pathogenic or disease-producing.

---

[1] *Die Milzbrand-impfung*, 1883.

From what has been said, it will be seen that bacteriology, as applied to disease, is dependent upon observation of, and experiments upon, living matter. Among phenomena of so complex a character as infectious diseases, simple observation goes but a very little way, and our knowledge of all the most important truths of bacteriology, as applied to these diseases, has been obtained by experimentation upon living animals.

Vivisection is necessary for a proper interpretation of the phenomena. But 'every now and again a loud outcry is raised against this method, partly from ignorance and partly from prejudice. Many—probably most—of the opponents of experiments on animals are good, honest, kind-hearted people, who mean well, but either forget that man has rights against animals as well as animals against man, or are misled by the false statements of the other class. These are persons who, blinded by prejudice, regard human life and human suffering as of small importance compared with those of animals, who deny that a man is better than many sparrows, and who, to the question that was put of old, "How much, then, is a man better than a sheep?" would return the reply, "He is no better at all." Such people bring unfounded charges of cruelty against those who are striving, to the best of their ability, to lessen the pains of disease both in man and also in animals, for they, like us, are liable to disease, and, like us, they suffer from it.'[1]

Without vivisection, the important researches of Pasteur, Koch, Klein, and others could not have been conducted; in fact, vivisection is absolutely necessary to ascertain the pathogenic nature of any microbe.

We now proceed to detail the general properties, etc., of microbes. All microbes contain two principal parts—a cell-wall or limiting membrane and a semi-fluid contents—the protoplasm. The cell-wall is composed of cellulose—a carbohydrate having the empirical formula $C_6 H_{10} O_5$. The protoplasm appears to vary somewhat in its chemical composition; for, in some microbes, this complex substance is devoid of sulphur and phosphorus, whereas in others, both of these elements are present. The protoplasm which is devoid of sulphur and phosphorus, has been termed mycoprotein by Nencki, and has entirely different reactions from the protoplasm containing sulphur and phosphorus.

Microbes are capable of giving rise to various products, such as acids, alkaloids, colours, enzymes, albumoses, etc. This property depends upon the present potentialities of the protoplasm (in each case), and the inter-relation of its various functions, and these again result from, or are modified by, the adjustment which takes place between an organism and its environment. For instance, the cholera bacillus grown on albumin produces toxines or alkaloids, and is pathogenic; on potatoes, it gives rise to a brown pigment and is chromogenic; while on sugar it produces butyric acid, and is consequently zymogenic or fermentive (Hueppe). The action of gases, heat, light, electricity, and various antiseptics have the power of altering the common properties of a microbe; but in every case the usual products, etc., are formed when the microbe is once more transferred to its natural mode of life. 'Every organism has more potentialities or modes of action than those which are actually in operation at any given time, and when the environment is changed one or other of these potentialities may come into action, replacing, more or less completely, a former one.' The extent of the powers of adaptation of an organism depend on its potentialities and their capacity of extension, and these vary, in each case, enormously, a view in perfect consonance with the results which experiments have already yielded.

---

[1] Dr. Lauder Brunton in *Nature*, vol. xliv. p. 331.

As microbes differ in their actions, they likewise differ in their dimensions; and, as a general rule, they vary from about 0·0005 mm. to 0·05 mm. in length or diameter, as the case may be. Dr. F. Cohn calculated that one bacterium (*Bacterium termo*) weighs 0·000,000,001,571 milligramme, or that six hundred and thirty-six millards of bacteria would weigh one gramme, or six hundred and thirty-six thousand milliards a kilogramme; and the late Professor J. Clerk Maxwell stated that the smallest organised particle visible under the microscope contains about two million molecules of organic matter.

The reproductive power of microbes is most prolific; and Cohn has calculated that a single microbe at the end of three days would have increased to nearly forty-eight billions, a mass which would weigh no less than 7500 tons. But this astounding rate of reproduction is kept in check by the limited supply of food, as well as by various circumstances which make the environment unsuitable for such a rapid rate of increase. 'As a consequence of their enormous fecundity, it will be readily understood that they are ubiquitous. Every surface teems with them; all natural waters are infested by them; even the skin of the most washed of mankind; even the moisture of the sweetest mouth harbours them by the million! One thing, however, they cannot stand, and that is boiling. Boil them or the stuff in which they are flourishing, and they cease to live—or, in other words, the liquid or solid substance so treated is sterilised. By means of sterilised nutriment we can test any object for the presence of microbes or bacteria, as they are sometimes called. We prepare a broth suitable for their nourishment, and sterilise it. If kept hermetically sealed (as are preserved vegetables and tinned meats), no microbes will appear in the broth. Touch the broth with any stick or stone, or add to it a drop of purest spring water, and it will, after a few hours, swarm with microbes and putrefy. This was the discovery of Theodore Schwann, also celebrated for his cell-theory. He showed fifty years ago that what we call 'putrefaction' is not the result of death, but of life. The unpleasant smell and the disintegration of dead bodies, whether of plants or animals, is entirely due to microbes—it is the accompaniment of their digestion. If you destroy all the microbes present by means of boiling heat, and then prevent the access of new microbes (which are blown about in the dust of the air), dead bodies never putrefy. Supposing that by the fiat of an omnipotent Being all microbes could be annihilated, the earth's surface would soon be covered with dead bodies remaining unchanged year after year, century after century. The seas and lakes would be choked with them, and we should have to use them for paving our roadways and building our houses. But worse than that, all the carbon and nitrogen which living things use in turn in their successive occupation of the earth's surface from generation to generation, would soon be tied up. There would be no food for the green plants: herbivorous creatures would cease to exist. The contemplation of these imaginable horrors gives us some notion of the part played by microbes in the order of nature.'

CHAPTER II

THE BACTERIOLOGICAL LABORATORY AND ITS FITTINGS

BEFORE describing the necessary apparatus, etc., for the proper investigation of bacteriological problems, we give a general account of the laboratories of the Royal College of Physicians, Edinburgh, and those of the Pasteur Institute, Paris: these being chosen as typical examples of bacteriological laboratories.

The Edinburgh Laboratory.—The ground floor of this laboratory, which is situated in Lauriston Lane, contains a workshop, stores, and a room set apart for experimental physiology. The latter is 32 feet long, 18 feet wide, and 14 feet high: it is fitted with tables for microscopical work; a respiration apparatus driven by water-power; recording apparatus; galvanometer and other electrical appliances; and sink and draining apparatus. 'On each microscope table, which is painted black and hard varnished, a white band about four inches broad is painted, four inches from the edge of the tables. Some of the tables, instead of being varnished, are covered with plate glass, painted as above on the under surface, and imbedded in felt. On these glass-covered tables the microscope stands

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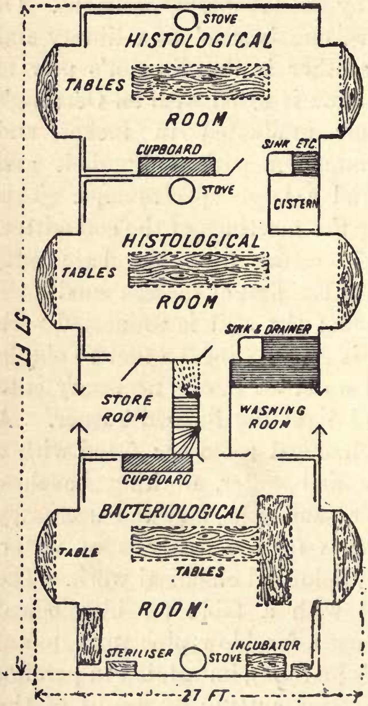
on a felt circle, to diminish the risk of breakage when the bell jar is lowered over the microscope.'

On the second floor there are five rooms, three of which are occupied by the laboratory assistant. Of the other two rooms, one is used as a library and museum; while the other is the director's private room. The former room is fitted with an Oërtling's balance, a barometer graduated in inches and millimetres, a thermometer with Fahrenheit and centigrade scales, and a large spectroscope. This room is also used for the meetings of the committee.

The third floor (counting from the basement) contains six rooms. 'The first of these, a small one, is used as a still-room; the still is connected with the water-pipe and is self-feeding, so that to obtain a supply of distilled water all that is necessary is to turn on the tap and light the Bunsen burner.' A second room—the chemical room—is fitted with a good supply of gas and water, working benches, evaporating chamber, sandbaths, and the necessary apparatus and reagents for the analysis of water, air, food, and for physiological chemical work. The next room is fitted with a table for histological work, but is chiefly used for blow-pipe work, metal injections (Cathcart's method), imbedding in paraffin and celloidin, and section-cutting by means of the microtome, etc. The same room is also used as a store for some of the glass apparatus. The next room is used as a store for chemical reagents. This is followed by another small histological room; and finally, a room is set apart for the estimation of urea, albumin, and glucose in urines.

The fourth and top story contains three well-lighted rooms (Fig. 1). The south room is the true bacteriological laboratory (Figs. 1 and 2), and is fitted with tables, microscopes, sterilisers, incubators, and the apparatus necessary for research in the various branches of bacteriology. The other two rooms on the top story are fitted for histological work.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STORE ROOM
WASHING ROOM
CUPBOARD
BACTERIOLOGICAL
TABLE
TABLES
ROOM
STERILISER
STOVE
INCUBATOR
57 FT.
27 FT

FIG. 1. PLAN OF BACTERIOLOGICAL LABORATORY, ETC.
(*Royal College of Physicians, Edinburgh.*)

The original cost for the whole equipment of the 'Edinburgh Laboratory' was only £830.[1] Of course this laboratory, being a public one, has been fitted for many workers; but a good private laboratory, suitable for one's own researches, can be fitted at less than a fourth of the above-mentioned amount.

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[1] See Dr. G. Sims Woodhead's paper in *Proc. Roy. Physical Soc., Edinburgh*, vol. ix. p. 521.

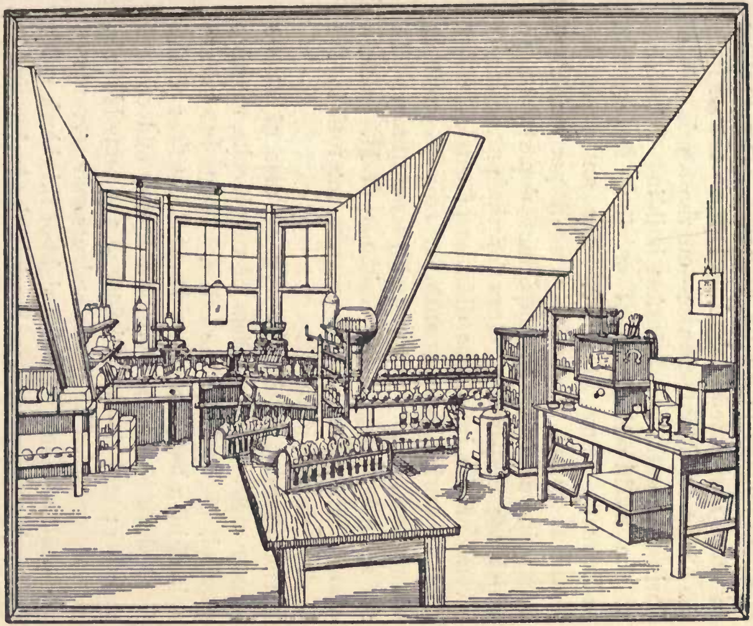

FIG. 2. THE INTERIOR OF THE BACTERIOLOGICAL LABORATORY.
(*Royal College of Physicians, Edinburgh.*)

The Pasteur Institute.—This celebrated institution (see frontispiece) is not simply an hospital for the treatment of persons suffering from hydrophobia or rabies, but is a building set apart for the study of micro-biology in all its branches. The Pasteur Institute is situated in the Rue Dutot—not far from the Cimetière Montparnasse—on the south side of Paris. It is the most perfect building of its kind in the world; the cost of erection, fitting, and endowment being £100,000. The anti-rabic department forms a relatively small portion, there being in addition an important department, in which are prepared vaccines for the prevention of several of the infectious diseases of cattle—*rouget de porc* (swine fever), anthrax, etc.—as well as laboratories, lecture-rooms, and a large library. In the same building is the residence of M. Pasteur, who naturally takes the greatest interest in the work of the institute.

The Pasteur Institute covers an area of 11,000 square metres, and consists of two blocks, running parallel, one behind the other. These blocks are united by a long corridor. On the first floor of the *front* block is a room used as a library and council chamber; and the second floor of the same block is entirely occupied by the attendants and servants of the establishment. On the right of this block is M. Pasteur's residence. The block in the *background* is divided into two wings, each about 25 metres long, and 15 metres from back to front. In the right wing, on the ground floor, are the rooms set apart for the anti-rabic treatment, and a laboratory in which the preparation of the virus is carried on. This laboratory is always maintained at a temperature of 23° C. In the left wing, on the ground floor, is a lecture-theatre for biological chemistry, a laboratory, and a room set apart for photographing microbes, etc. At the end of the block (*i.e.* at the back) are two rooms (one on each side of a central corridor) used as aquaria. The remaining portion of this block is occupied as a store-room and a general laboratory; the latter being used for the preparation of the various cultivating media, glass-blowing, etc. The second story is, likewise, divided into two halves; on the left is the micro-biological department, and on the right that of practical biology. On the same floor there is a large laboratory fitted with sterilisers, incubators, evaporating chambers, etc.: in fact, this room is used for the growth of all kinds of microbes. Joining this room is a smaller laboratory, out of which one steps into the museum. In addition, there is a chemico-biological laboratory and a lavatory. The third story of the rear block comprises two series of rooms, which are all used for research; the left wing is occupied by the department of applied or practical bacteriology, while the right wing is devoted to the study of comparative micro-biology or bacteriology. Each of these departments is fitted with incubators, sterilisers, and other bacteriological apparatus.

In each of the departments of the institute there is a director's private room and laboratory.

Besides the two main blocks there are separate buildings, etc., in the grounds of the Institute. Among these are the cages for the accommodation of animals; a special house for the reception of dogs; stables, etc., for large animals; a rabbit-house; a run, etc., for hens; and an aviary. All these places are kept in a state of perfect cleanliness. This important establishment would not be complete without a crematory; this consists of two large furnaces, situated in one corner of the grounds, which are used for destroying all useless animal matter.

The Pasteur Institute accommodates fifty workers, and is open to foreign as well as French scientific and medical men. Besides being an institute for research, it is also used for the instruction of pupils in both general and special methods of bacteriological investigation.

The above is only a general account of the Pasteur Institute; but the reader desirous of obtaining fuller information is referred to the *Annales de l'Institut Pasteur*, 1889.

Having given a description of the Edinburgh and the Paris laboratories, we now proceed to describe the various apparatus and appliances used in the study of bacteriology.

The Microscope.—In our experience the best microscopes suitable for the study of 'les infiniment petits' are those made by Carl Zeiss, of Jena (Fig. 3). These instruments are monocular microscopes, and consist of the usual parts—the stand, eye-pieces, and objectives. Very few workers in bacteriology use the binocular microscope, because there is a great loss of light, and the definition of high-power objectives is impaired when this instrument is used. It is believed by some that when the monocular microscope is used continuously the eyes are apt to become fatigued. The student should learn to keep both eyes open when working with the monocular microscope. There are several ways by which this may be effected. One is by having a black sheet of paper near to the second eye; another plan is to put the hand before the eye. Perseverance is all that is needed. One evening is quite enough to make any one skilful, if he is determined to succeed. The student need no more fear seeing things on the table with the second eye than seeing the crown of his head, unless he is training for drawing objects on the table by

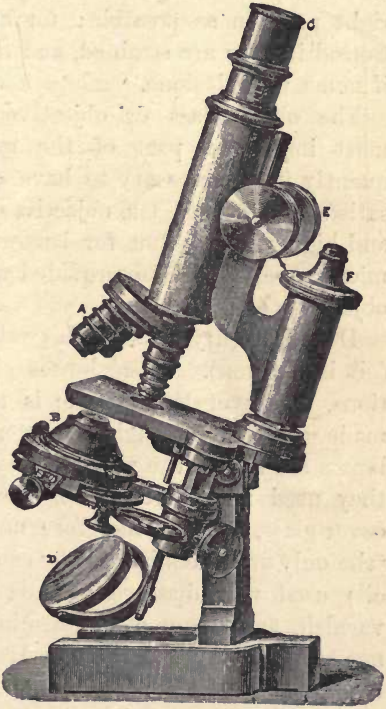

FIG. 3. ZEISS' MICROSCOPE.

means of a camera lucida from the instrument, whilst with one eye he looks at the object, and with the other draws the figure. A very little reflection will convince any one how desirable it is to keep the nerves of the eye as nearly in their right position as possible; for an undue strain is caused if they are strained, and the sight is injured if much work is done.

The object-glass or objective (Fig. 3A) is the most important part of the microscope; consequently it is necessary to have good lenses to do satisfactory work. The objectives are known as low and high powers—but for bacteriological work, the microscope should be provided with the following objectives (Zeiss'):—

D and E (dry lenses), J (water immersion), $\frac{1}{12}$ (oil immersion). Zeiss' lenses give perfect definitions, and everything there is to be seen can be made out with the highest powers. Oil-immersion lenses are taking the place of water high powers, as they need no correction for the thickness of the cover-glass, and are therefore much easier to use; 'the only drawback is that the essential oil (*e.g.* cedar oil) used will dissolve Canada balsam, Dammar varnish, and many of the sealing fluids, and it is necessary to cover them with Hollis' glue, which is not acted on by cedar oil.' Of Zeiss' high powers the $\frac{1}{12}$ oil-immersion lens is the best, and may be thoroughly recommended for bacteriological research. Oil-immersion lenses possess far greater brilliancy and definition than the water and dry lenses [such as Zeiss' K and L (water), and F (dry)]. In using oil-immersion lenses, a drop of cedar oil is placed on the front glass, the lens in use is then lowered on to the slide until contact is made. The lens is then focussed by the fine adjustment (Fig. 3F) until the object is seen sharply defined.[1]

It is desirable that the bacteriologist's microscope should be fitted with a revolving triple nose-piece (Fig. 3A); by this means three objectives (of different magnifying power) can be brought successively into position, without unscrewing.

The eye-piece or ocular is also an essential part of the microscope (see Fig. 3C); and for bacteriological research, the *whole* (five in number) of Zeiss' huyghenian eye-pieces are recommended. The following table shows the magnifications of Zeiss' objectives and eye-pieces with a tube of 155 millimetres in length—*i.e.* the Continental microscope with a short tube:—

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[1] Zeiss no longer makes the $\frac{1}{18}$ oil-immersion objective; this lens has now been superseded by the introduction of a new series of objectives—the apochromatic lenses—made of the new glass. These lenses are said to excel the ordinary objectives, by giving almost perfect achromatism and sharpness of image over the whole visual field (see Abbé's paper in *Sitzungsberichte d. med.-naturw. Gesellschaft zu Jena*, 1886).

EYE-PIECES.		I.	II.	III.	IV.	V.
Dry objectives.	a_1	7	11	15	22	
	a_2	12	17	24	34	
	a_3	20	27	38	52	
	a		4–12	7–17	10–24	
	aa	22	30	41	56	75
	A, AA	38	52	71	97	130
	B, BB	70	95	130	175	235
	C, CC	120	145	195	270	360
	D, DD	175	230	320	435	580
	E	270	355	490	670	890
	F	405	540	745	1010	1350
Water-immersion objectives.	G	260	340	470	640	855
	H	320	430	590	805	1075
	J	430	570	785	1070	1430
	K	570	760	1045	1425	1900
	L	770	1030	1415	1930	2570
Oil-immersion objectives.	$\frac{1}{8}$	260	340	470	640	855
	$\frac{1}{12}$	380	505	695	950	1265
	$\frac{1}{18}$	605	810	1110	1515	2020

Zeiss' J objective is equal to an English $\frac{1}{15}$ inch, while his B, C, D, DD, E, and $\frac{1}{12}$ oil-immersion are equal to an English 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{6}$, $\frac{1}{8}$, and $\frac{1}{30}$ inch respectively. The medium objectives are issued in two different forms, with a greater or less aperture according to the purpose for which they are required. Those with a large aperture (distinguished by double lettering), possess with equally perfect definition a considerably higher resolving power, and permit of greater magnification being obtained by the use of the stronger eye-pieces. Nevertheless, the working distance in BB, CC, DD, although relatively large, is perceptibly less than in the corresponding series of smaller aperture, and the former are more sensitive to differences in thickness of the cover-glass and object than the latter. Therefore, B, C, and D are recommended as the more suitable for working glasses in histological and anatomical research, particularly when the next stronger dry lens is available for higher magnification.

The magnifications of the English objectives and eye-pieces with a ten-inch tube (*i.e.* the English microscope with a long tube), are given in the following table :—

OBJECTIVES.	EYE-PIECES.			
	A	B	C	D
4 inch.	10	14	28	40
3 ,,	20	27	40	52
2 ,,	30	40	60	75
1 ,,	60	80	120	150
$\frac{2}{3}$,,	75	100	150	190
$\frac{1}{2}$,,	100	133	200	250
$\frac{4}{10}$,,	170	227	350	440
$\frac{1}{4}$,,	254	333	500	625
$\frac{1}{5}$,,	270	360	540	675
$\frac{1}{8}$,,	450	600	900	1125
$\frac{1}{10}$,,	500	666	1000	1225
$\frac{1}{12}$,,	700	940	1350	1640

The above will serve as approximately correct tables for ordinary work, but if the *exact* magnifying power of any objective is required it must be specially tested.

The proper illumination of microscopic objects is of the highest importance, and that suitable for one class may be altogether unfit for another. Daylight is the best light to use for bacteriological work; but if one is working at night or in the winter, a paraffin lamp is required. It is essential that the flame should be steady and of moderate size. Parallel rays may be advantageously thrown on the mirror (Fig. 3D) of the microscope by means of a bull's-eye condenser, placed so that the flame is nearly in the focus. For comparatively low powers, a flat or concave mirror may be used to reflect the light, but for higher powers it is essential that the light should be concentrated by means of an Abbé's substage condenser (Fig. 3B). This condenser, first described by Professor Abbé (of the University of Jena),[1] is of very short focus, and collects the light reflected by the *flat* mirror into a cone of rays of very large aperture, and projects it on the object. Abbé's condenser is focussed on the object by coarse and fine adjustments. 'When the whole field is to be examined, the lamp is used with the whole breadth of the flame, but when a small portion is to be specially examined with a high power, it is necessary to turn the lamp so that the edge of the flame is presented, by which the light is very much intensified. The correct distance at which to place the lamp can only be found out by practice. A piece of blue glass should be interposed between the lamp and the condenser: this can be done by having it fitted into the condenser or by having a separate stand; different shades of blue will be found useful for various objects.

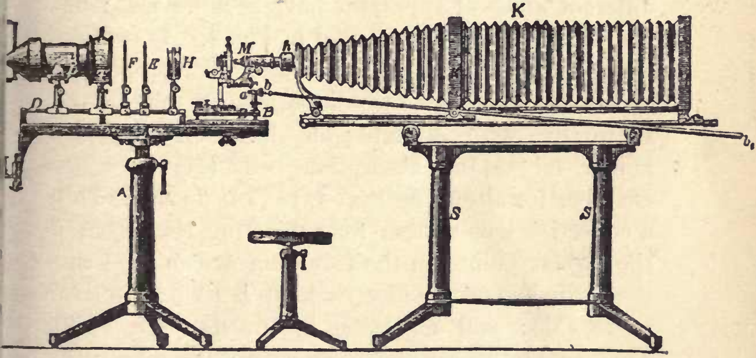

FIG. 4. MICROPHOTOGRAPHIC APPARATUS.
(*After Carl Zeiss.*)

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[1] *Archiv für Mikr. Anat.* vol. ix. p. 496.

The blue colour is a great help to the eyes, and also throws up the stained specimen with more distinctness.'

Microphotographic Apparatus.—The application of photography as a means of illustrating microscopic preparations has been, on the whole, successful. Koch, Crookshank, Van Ermengem, and others, have produced beautiful photographs of microbes and sections of diseased tissues. For this purpose many different kinds of apparatus have been devised; but one of the best is represented in Fig. 4. It consists of two tables (A and S) for the microscope and camera respectively; two diaphragm carriers (E and F) for use with sunlight; an electric lamp (C); a holder for taking absorption cells (H); a water chamber for absorbing heat rays (T); a camera (K); a collective lens-system for projecting the image of the carbon points on the focussing screen (L)—this is required when the electric lamp is used; a microscope (M); and focussing apparatus (*a*, *b*, *b'*, *h*). This apparatus can be used with sunlight,[1] lime-light, electric-light, and lamp-light. For microphotographic purposes, microscopic preparations are best when stained yellow, black, or brown, and mounted in either Canada balsam (dissolved in xylol) or a saturated solution of potassium acetate. Several authors have recommended the use of the isochromatic dry-plates, and first-class photographs have been obtained by them.[2]

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[1] When sunlight is used, a heliostat is also necessary.

[2] See Crookshank's *Photography of Bacteria*; and Van Ermengem in *Bulletin de la Soc. Belge de Microscopie*, No. 10, 1884.

Another method for obtaining illustrations of microscopic preparations is by means of the camera lucida. Among the best of these instruments, suitable for bacteriological purposes, are those of Zeiss and Nachet. 'Combined with the use of a micromillimetre objective, the camera lucida affords also a simple method for the measurement of bacteria.'

The third and last method for obtaining illustrations of microscopic preparations is drawing by hand. If a white piece of card-board or smooth drawing-paper is fixed at the same level as the stage of the microscope; by keeping both eyes open—one for looking at the object through the microscope, and the other for looking at the piece of card-board —an image of the object is seen on the card, which can be readily traced with a pencil. For drawing bacteria, etc., no pencil is so well adapted as Windsor and Newton's HHHH; the blacklead being brought to its final point by gentle rubbing on the surface of the finest ground glass, or, better still, a very fine hone. For inking the pencil drawings, the finest etching pens should be used—perhaps the best are those made by Joseph Gillott; and the same maker's No. 303 is also a very fine-pointed pen. In addition to the pencils and pens —Indian-ink, water-colours, and brushes are necessary. With practice and patience, very accurate drawings of microscopic preparations can be made by hand.

Dissecting Instruments.—For the dissection of diseased organs, tissues, etc., certain instruments are necessary. Figs. 5 and 6 represent various kinds of scalpels, microscopic needles, knives, scissors, and forceps; and Fig. 7 illustrates a very useful form of dissecting microscope.

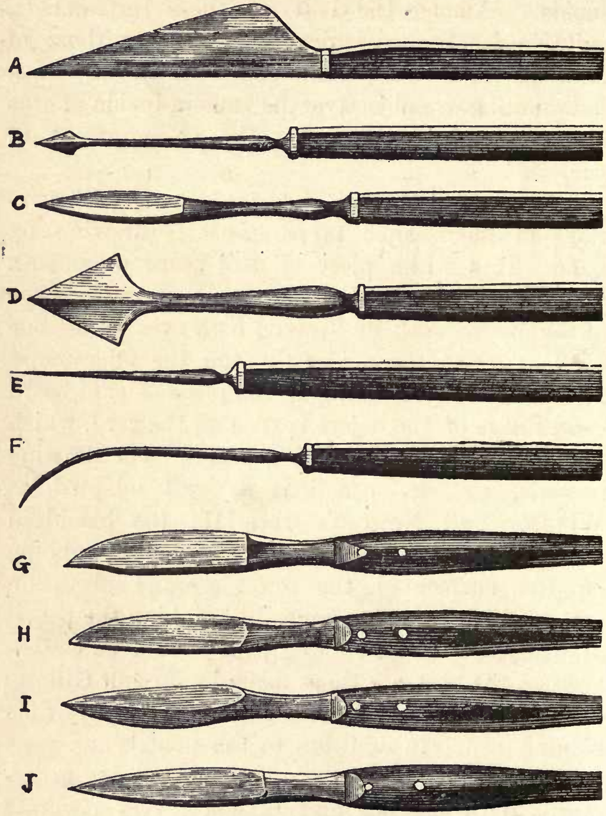

Fig. 5. Dissecting Knives and Needles.
A to F are used in microscopic dissections.
G to J are used in ordinary dissections.

The mode of carrying out a dissection for bacteriological purposes is as follows: Animals either artificially inoculated with pathogenic microbes or those naturally suffering from infectious diseases should be dissected as soon after death as possible. In dissecting, every precaution must be adopted to exclude putrefactive or other microbes. The dissection should be performed in a perfectly still

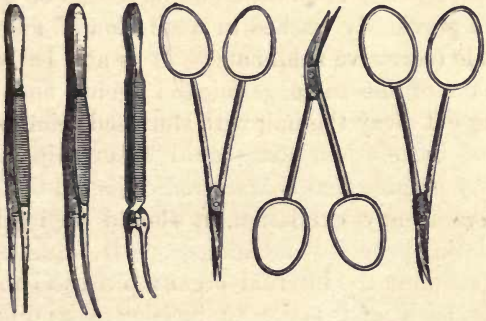

FIG. 6. DISSECTING SCISSORS AND FORCEPS.

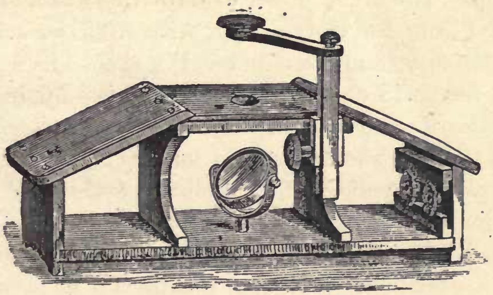

FIG. 7. DISSECTING MICROSCOPE.

room with closed doors; and the instruments used in the dissection must be previously sterilised in the hot-air steriliser or the Bunsen flame. The animal under examination (*e.g.* a mouse, rabbit, guinea-pig, etc.) is pinned out on a slab of gutta-percha previously washed in a solution of mercuric chloride (corrosive sublimate). It is now bathed in a stream of the same germicidal agent; and after having cut away the hair with sterilised scissors, the seat of inoculation, etc., should be examined first, and any pathological characteristics should be noted. If there is any exudation, it should be used for inoculating purposes and microscopical examination.

To examine the internal organs, place the animal on its back and make an incision extending (if necessary) from the abdominal to the thoracic region. The organ under examination should be removed from the body-cavity, with sterilised scissors and forceps; and after removal it should be washed with mercuric chloride. The organ is now incised, and the fluid, or a portion of the organ itself (*i.e.* from the cut) should be used for inoculating various cultivation media. If the blood of the animal is required, it is best obtained from a vein by making an incision with sterilised scissors, and then inserting a sterilised capillary pipette or a platinum needle. The blood so obtained should be examined microscopically, and various cultivation media inoculated with it. If the cultivations are contaminated by the presence of other microbes, fractional plate-cultivation must be resorted to, in order to isolate the pathogenic microbe.

After dissection, the organs, etc., may be preserved in absolute alcohol, *i.e.* if they are required for future examination; and all useless matter should be destroyed, and, finally, the hands, instruments, and table disinfected.

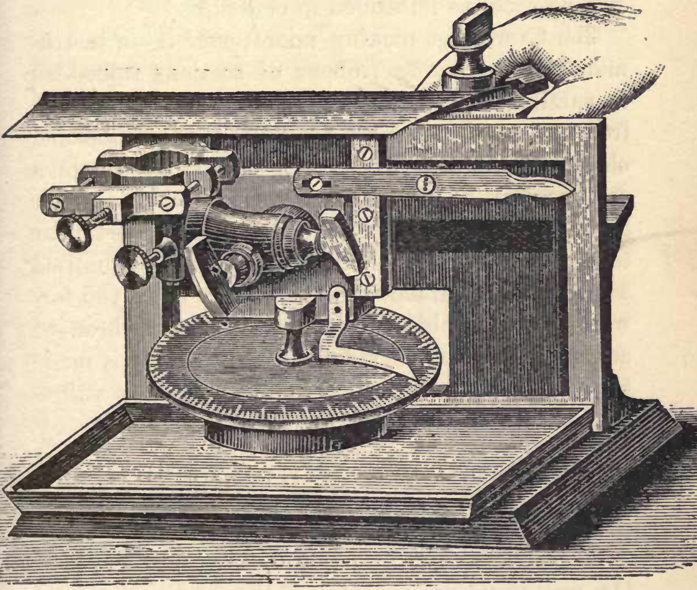

FIG. 8. SCHANZE'S MICROTOME.

Such is the mode of carrying out a dissection on a dead animal; but to obtain microbes from the living animal or from man, these may be isolated from pus and other discharges, or from the blood. These fluids must be obtained with all the necessary precautions to prevent external microbes gaining an entrance.

Microtomes.—These instruments are used for cutting sections of organs, tissues, etc.; and there are many forms in use. Fig. 8 represents Schanze's microtome, and it is a most useful instrument for cutting sections imbedded in celloidin.

The Cambridge rocking microtome[1] is an instrument for producing ribbons of sections imbedded in paraffin. The razor is supported and clamped in front of a brass tube containing the imbedded object. This tube fits tightly on to the end of a cast-iron lever; and is made to slide backwards or forwards so as to bring the imbedded object near to the razor. By an arrangement of pivots, milled screws, and a milled wheel, the lever is moved forwards, and the object to be cut is therefore brought across the edge of the razor: when the lever is made to move backwards the section is cut. The values of the teeth on the milled wheel are as follows:

1 tooth of the milled wheel = $\frac{1}{40000}$ of an inch = ·000625 mm.
2 teeth ,, ,, = $\frac{1}{20000}$,, = ·001250 mm.
4 ,, ,, ,, = $\frac{1}{10000}$,, = ·0025 mm.
16 ,, ,, ,, = $\frac{1}{2500}$,, = ·01 mm.

On working this microtome the sections should adhere together so as to form a ribbon. The working of this instrument requires very little skill on the part of the operator; consequently it is to be recommended to those who require very thin sections of diseased organs, etc. Dr. Sims Woodhead has somewhat modified the Cambridge rocking microtome by adding a solid end to the brass tube into which 'dies' of various sizes, with roughened surfaces, can be screwed. 'This does away with the inconvenience of having to "melt in" the imbedded tissue into the tube. A dozen of the dies may be used, and to each of these a piece of tissue may be fused, and kept ready for cutting at any time.'

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[1] Made by the Cambridge Scientific Instrument Company, St. Tibb's Row, Cambridge.

Besides the microtomes just mentioned there are those of Körting, Reichert, and Jung, which are principally used in France and Germany. When tissues are to be examined in the *fresh* state, either Roy's or Williams' freezing microtome should be used for section-cutting. In the former instrument the tissue is frozen by means of an ether spray; while in the latter the frozen tissue is produced by a mixture of ice and salt. There is no doubt that Roy's microtome is the better instrument of the two, as the freezing of the tissue only occupies from thirty to forty seconds; and this microtome may also be used for cutting objects imbedded in paraffin and not requiring freezing—in other words, the instrument can be used both as a freezing and a non-freezing microtome.

Tissues imbedded in paraffin or a mixture of white wax and olive oil may be cut by hand with a hollow-ground razor. The razor is dipped in dilute alcohol and then drawn diagonally across the mass (containing the specimen) with a steady sweep. Before cutting each section the razor should be dipped in dilute alcohol. 'Great care is required in cutting sections by hand, to hold the razor firmly yet lightly, so as to cut them thin and at the same time even, and this cannot be done without a great deal of practice.' The author has, in his possession, sections of the human brain varying from the $\frac{1}{3000}$ to the $\frac{1}{4000}$ of an inch in thickness which were hand-cut by Dr. E. Palmer, who was formerly the resident physician to the Lincoln County Asylum, Lincoln. To obtain such sections requires skill and practice, therefore it is better to use the microtome.

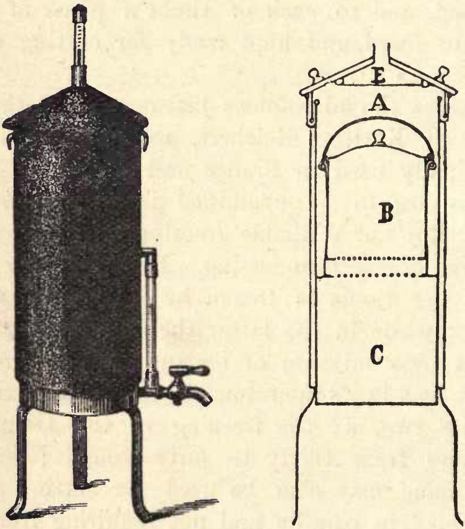

FIG. 9. KOCH'S STEAM STERILISER.

Sterilisers.—In a study like bacteriology, all vessels, instruments, etc., used in the cultivation of microbes, must, before use, be rendered perfectly sterile. It cannot be too firmly impressed upon the mind that the only way to obtain pure cultivations of microbes, is the complete sterilisation of all vessels and instruments used by the experimenter. For the accomplishment of this object steam, hot-air, Bunsen or spirit flames, and germicides are used as sterilising agents.

Fig. 9 represents Koch's steam steriliser; and is used for sterilising test-tubes, flasks, and for cooking potatoes. It is a cylindrical vessel of stout tin plate, with a copper bottom, provided with a conical lid, brass tubulure for the insertion of a thermometer, a grating, water gauge, tap, and a receiver with perforated bottom for cooking potatoes (*b*). The cylinder (which is 20 in. high and 10 in. diameter) is divided into two compartments (*a* and *c*). The lower one contains boiling water, while the steam therefrom passes into the upper or sterilising compartment. The cylinder is heated from below by a Bunsen's or Fletcher's burner. Steaming is usually kept up for from fifteen to twenty minutes; and this operation is repeated on three successive days each time for twenty minutes. By such steaming the various cultivation media, etc., are rendered sterile, *i.e.* free from microbes. A later form of this steriliser contains three compartments instead of two. Two of these are used as sterilising compartments, while the lowest one contains the boiling water, which is always kept at a constant level. Both forms of Koch's steriliser are covered externally with felt.

The so-called steam digesters or 'autoclaves' are chiefly used in France. They are made of stout copper, and are used for sterilising *sealed* flasks (containing bouillon) under pressure. The temperature in these digesters often rises as high as 120° C.

Besides the above-mentioned steam sterilisers, there are those of Herrmann and Hirschberg, Ostwalt, Muencke,[1] and Woodhead.[2] All these are useful instruments, and are to be recommended for the bacteriological laboratory.

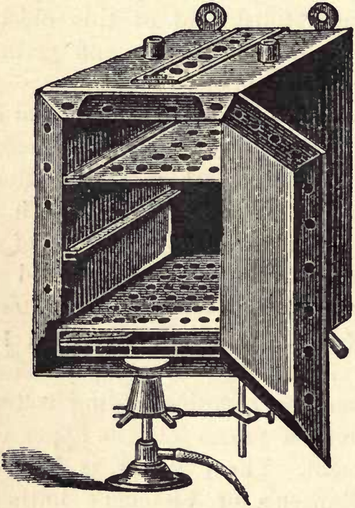

FIG. 10. HOT-AIR STERILISER.

Two forms of *hot-air* sterilisers are represented in Figs. 10 and 11 respectively, and are used for the sterilisation of flasks, test-tubes, cotton-wool, etc. The former consists of a double wall of sheet-iron, and the inner dimensions are 12 in. × 10 in. × 10 in. It is heated by a gas-burner, and is made to hang against the wall of the laboratory.

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[1] Dingler's *Polytechnisches Journal*, 1885, Bd. 257, p. 283.

[2] *Proceedings of Royal Physical Society of Edinburgh*, vol. ix. p. 534.

The steriliser represented in Fig. 11[1] is either heated by paraffin-oil or by gas. It consists of a sheet-iron chamber, provided with shelf containing

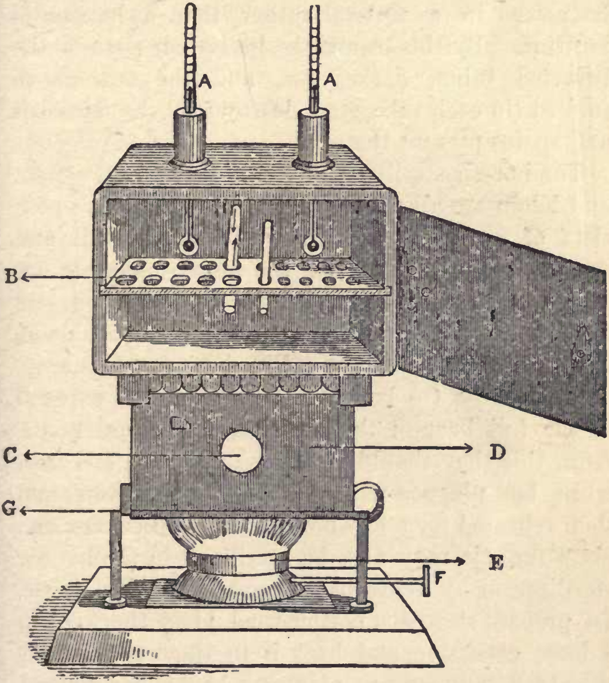

FIG. 11. HOT-AIR STERILISER HEATED BY PARAFFIN OIL OR GAS. (*Devised by Mrs. A. B. Griffiths.*)
A, Thermometers. B, Copper Shelf with Holes of different sizes. C, Mica Window. D, Iron support for Oven over Flame. E, Paraffin Oil Lamp. F, Screw to raise Wick. G, Wire Gauze.

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[1] First described by the author in the *Proceedings of the Royal Society of Edinburgh*, vol. xiv. p. 105.

a series of holes of different sizes; by this means the tubes or flasks are placed in a vertical position in the steriliser. It may be stated that all good hot-air sterilisers should allow the tubes to be placed in a vertical rather than a horizontal position. By this means the heated air rises in the inverted tubes, flasks, etc., and the current so formed (in each tube, etc.), destroys all the microbes and spores present therein.

The hot-air sterilisers of Koch, Muencke, Pasteur, and Klein are all good sterilisers. Dr. Klein's consists of an iron chamber with double wall and double folding-doors. In the inner chamber are placed the test-tubes in a horizontal position, and the cotton-wool above them. After closing the doors the steriliser is heated by a Fletcher's gas-burner.

'Test-tubes (to be sterilised) should be exposed to the full heat of the chamber for several hours. After this they should be taken out of the steriliser while hot, plugged with sterilised cotton-wool, and then reheated for a few hours longer. Beakers and glass funnels may also be sterilised in the hot-air steriliser, or by being heated over a Bunsen flame. To prepare sterilised cotton-wool, place the wool in a loose condition, and heat it in the hot-air steriliser to a temperature of about 150° C. for several hours on several successive days.'[1] Over-heating the cotton-wool in the hot-air steriliser to the above temperature until singed has proved invariably and absolutely safe for all cultivations.

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[1] See Dr. A. B. Griffiths' book, *Researches on Micro-Organisms*, p. 14 (Baillière & Co.).

To use cotton-wool, flasks, and tubes disinfected by prolonged steeping in alcohol, carbolic acid solution, and other chemicals, is not absolutely reliable; and many failures have been the results of such methods of sterilisation. Therefore, 'it cannot be too thoroughly insisted on that the flasks and test-tubes, and especially the cotton-wool used as plugs for the vessels, should be thoroughly sterilised by over-heating, for cultivations are as often contaminated by this not being properly carried out as by the non-sterility of the nourishing fluids or the accidental entrance of organisms from the air.'

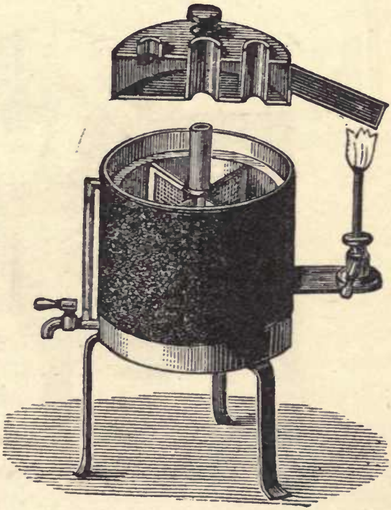

FIG. 12. SERUM STERILISER.

For the sterilisation of scalpels, forceps, platinum needles, etc., the Bunsen flame is the best way of cleansing them; but, unfortunately, the naked flame is most destructive to the blades of scalpels; to obviate this, Israël's case was devised. It is a sheet-iron box (with lid), in which the scalpels, etc., are exposed to a temperature of 150° C. in the hot-air steriliser for an hour or so. By this device the blades are not injured.

For the preparation of solidified sterile blood serum two pieces of apparatus are necessary; these are represented in Figs. 12 and 13. Fig. 12 is the serum steriliser, and consists of a double-walled cylinder, 13 inches in height and 11 inches in diameter, made of stout tin, with a copper bottom. This cylinder is provided with a double-walled lid, having a tubular prolongation of stout copper, tap, and gauge: the whole being surrounded with thick felt. The apparatus is divided internally into four compartments; and into these are placed the test-tubes, or glass capsules, containing the blood serum. Between the two walls of the cylinder is a layer of water, which is heated from below; while the water

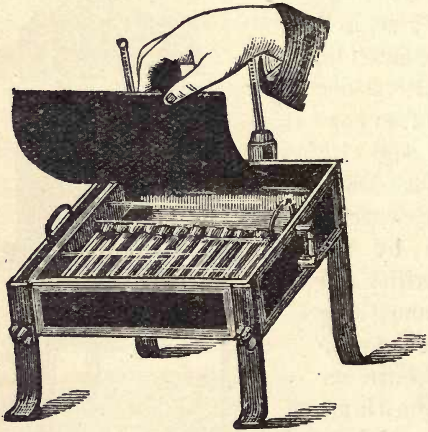

FIG. 13. SERUM INSPISSATOR.

in the lid (*i.e.* between the two walls) is heated by means of the prolongation (see Fig. 12). It will be seen that the whole apparatus is essentially a hot-water jacket. The temperature of the steriliser should be maintained for an hour at 60° C. on five or six successive days. By this means the fluid serum is completely sterilised, but it is not solidified. To solidify the serum the piece of apparatus represented in Fig. 13 is required. It is a double-walled case, also made of stout tin (13½ in. long × 13½ in. wide × 4½ in. deep). It is provided with a copper bottom, glass cover, water-gauge, and thermometers; there is also an arrangement by which this inspissator, as it is called, can be fixed at the angle required; this being necessary to give the serum a sloping surface. The tubes, etc., containing the sterile but fluid serum, are placed in the inspissator; and this apparatus (like the serum steriliser) containing water (between the two walls) is heated from below. To coagulate the serum, and to solidify nutrient agar-agar, a temperature between 65° and 68° C. should be maintained; but as soon as solidification takes place the tubes should be removed from the inspissator. Solidified blood serum is used for the cultivation of *Bacillus tuberculosis, Bacillus mallei,* and a few other microbes; but we shall refer, in detail, to the various cultivation media and the methods of cultivation, in the next chapter.

Incubators.—Many microbes are capable of being cultivated at the ordinary temperature of the laboratory; but certain microbes require a higher temperature for their proper development and multiplication. For the latter purpose various ovens or incubators have been devised. One of the most

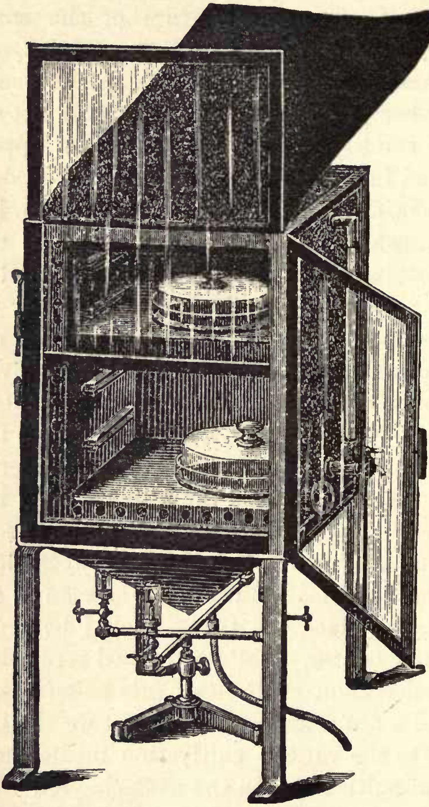

FIG. 14. BABÈS' INCUBATOR.

convenient forms is the incubator of Dr. Babès (Fig. 14). It consists of a rectangular, double-walled chamber, covered on five sides with felt, but in front the felt forms a loose flap, which can be raised. The interspace between the two walls is filled with water, which is heated from below. The incubator has two glass doors, a moveable shelf, a water-gauge, and a gas-regulator.

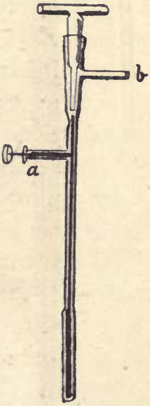

Fig. 15.
Reichert's Regulator.

Among other good incubators are those of Pasteur,[1] Rohrbeck,[1] Klein,[2] Gautier, Abel, D'Arsonval, and Hueppe. Whatever form of incubator is preferred, it is essential that it should be provided with a gas or heat regulator. The acting agent in most regulators of this description is either a membrane, mercury, or electricity. Tieftrunk's, Giroud's, and Elster's are membrane gas-regulators; Reichert's, Page's, Schütz's, Fraënkel's, and Meyer's are mercury heat-regulators; and Schlösing's is a membrane heat-regulator. Fig. 15 represents Reichert's mercury heat-regulator. It is a tube with two lateral arms (*a* and *b*); the upper portion of which is extended into a funnel-like arrangement, bearing the arm *b*. Into this funnel-like opening fits a hollow T piece. 'One arm of the T piece is open, and connected with the gas supply; the vertical portion terminates in a small orifice, and is also provided with a minute lateral opening.' The tube and arm *a* contain mercury. Reichert's regulator is fixed into the roof of the incubator, so that its lower portion projects either into the water chamber or into the interior of the incubator. 'When the incubator reaches the required temperature, the

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[1] For figures of these incubators see Dr. Griffiths' *Researches on Micro-Organisms*, pp. 19 and 20.

[2] See Dr. Klein's *Micro-Organisms and Disease* (3d ed.), p. 15.

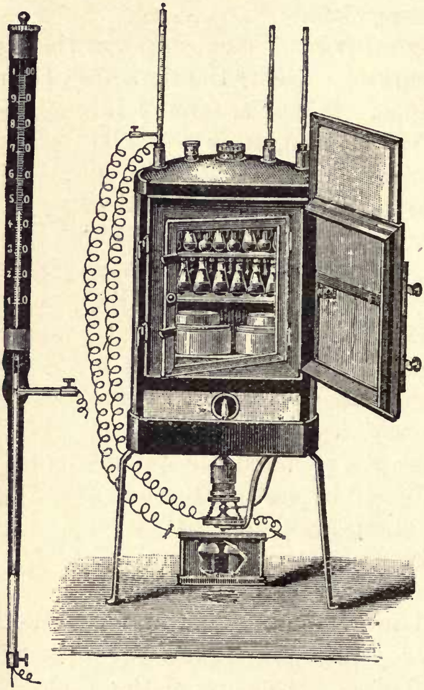

FIG. 16. KARL ABEL'S INCUBATOR.
(With thermo-electric regulator.)

mercury is forced up by means of the screw in the lateral arm, until it closes the orifice, at the extremity of the vertical portion of the T piece. The gas which passes through the lateral orifice is sufficient to maintain the apparatus at the required temperature. If the temperature of the incubator falls the mercury contracts, and the gas passing through the terminal orifice of the T piece, increases the flame of the burner, and the temperature is restored.' Page's regulator resembles somewhat the regulator just described: both are simple and useful forms for the bacteriological laboratory. By such devices the various incubators may be maintained at a temperature which is almost constant; the slight differences (say of one or two degrees Centigrade) are due to the variations in the pressure of the gas supply; but this inconstancy is remedied by first passing the gas through a pressure-regulator (such as Moitessier's).

In addition to the above-mentioned regulators, there are two forms which are worked by the agency of the electric current. Babès'[1] and Abel's[2] are thermo-electric regulators; the latter being represented in Fig. 16. These are useful regulators; but for general work those of Reichert and Page are specially recommended.

Cultivation Tubes, etc.—Fig. 17 represents an important series of glass tubes, flasks, etc., used in the cultivation of microbes in liquid media. These are first carefully washed with soap and water, then with a boiling solution of potassium permanganate, to which a few crystals of oxalic acid are added. They are then rinsed with distilled water, and are allowed to drain on a rack for some time, after which they are carefully plugged with cotton-wool, care being taken that the wadding inside the neck is perfectly smooth and firm, the tuft outside being large enough to overlap well the lip of the test-tube (Woodhead). They are

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[1] *Centralblatt für Bakteriologie und Parasitenkunde*, 1888.

[2] *Ibid.*, 1889, p. 707.

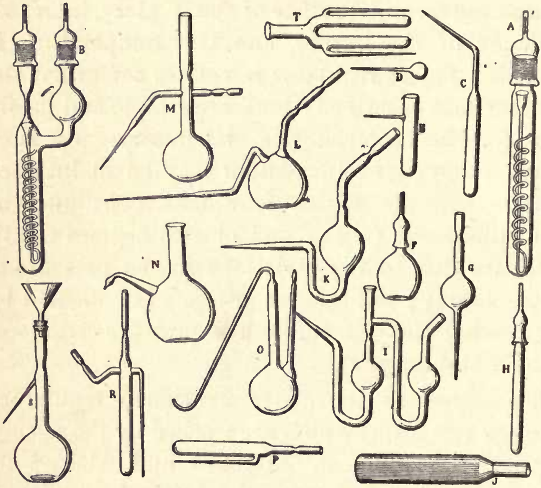

FIG. 17. CULTIVATION TUBES, FLASKS, ETC.

A, Gayon's Tube. B, Gayon and Dupetit's Tube. C, Chamberland's Tube. D, Sternberg's Bulb. E, Aitken's Tubes. F, Pasteur's Flask with Cap. G, Pasteur's Bulb Pipette. H, Pasteur's Test-tube. I, Miquel's Double Tube. J, Lipez's Tube. K, Miquel's Bulb Tube. L, Pasteur's Pipette Flask. MO, Pasteur's Flasks. N, Lister's Flask. P. Chamberland's Pipette. R, Duclaux's Tube. S, Miquel's Filter Flask. T, Pasteur's Double Tube.

then ready for the nutrient fluid and subsequent sterilisation.

Fig. 17 F and H represent Pasteur's flask and tube, both of which are provided with caps. The narrow portion of each cap contains a plug of cotton-wool. De Freudenreich's flask is somewhat similar to that of Pasteur. These are used for the cultivation of microbes in bouillon. In Pasteur's pipette flask (Fig. 17 L), the tube above the bulb is contracted twice, and on either sides of these contractions there are plugs of cotton-wool. The portion below the bulb is bent twice and is drawn out to a capillary point. The flask is charged with bouillon and inoculated by aspiration ; and then the capillary point is sealed in the Bunsen flame. Miquel's[1] bulb tubes (Fig. 17 K and I) are similar devices.

The tubes and flasks T, M, R, P (Fig. 17) are provided with lateral arms drawn out to fine points, and with necks plugged with cotton-wool. They are filled by aspiration and are convenient for storing sterilised bouillon. 'The sealed end of an arm is nipped off with sterilised forceps, the sterile bouillon aspirated into each limb, and the arm again sealed in the flame; a series of such tubes and flasks can be arranged upon a rack on the working table.'

Sternberg's bulbs (Fig. 17 D) are generally kept in stock in the bacteriological laboratory. They are readily prepared by blowing a bulb on a piece of glass-tubing, and then drawing the tube out to a fine point which is hermetically sealed. To fill a bulb, it is first slightly heated, then the sealed point nipped off, and the open end dipped beneath the surface of the culture fluid. As the bulb cools the fluid is drawn into it. The neck of the bulb is again sealed, and the fluid contained therein is sterilised by repeatedly boiling the bulb in a water-bath. It is then placed in an incubator for three or four days. If the contents remain transparent and clear, there is no doubt that the fluid has been properly sterilised. Many of these bulbs, containing sterilised bouillon, should be kept in stock.

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[1] *Les Organismes Vivants de l'Atmosphère*, 1883.

It may be mentioned that Chamberland's tube (Fig. 17 C) is filled and sterilised in the same way as Sternberg's bulb.

Sir Joseph Lister's flask (Fig. 17 N) is used for the storage of culture fluids. The fluid is introduced into the flask, the neck plugged with cotton-wool, and the fluid sterilised by repeated boiling. When a portion of the sterile fluid is required, all that is necessary is to pour it through the lateral arm of the flask: this is done by simply tilting the flask. When the flask regains its erect position a drop of the fluid remains behind in the fine opening of the arm; and thereby prevents the regurgitation of unfiltered air. After the removal of a portion of the fluid, a cap of cotton-wool is tied over the lateral opening, and the residue in the flask is kept for future use.

Aitken's tube (Fig. 17 E) is a modification of the ordinary test-tube. It has a lateral arm whose extremity is hermetically sealed. The nutrient fluid is introduced through the open end of the tube, which is then plugged with cotton-wool. The fluid is sterilised by heating in the usual way; and is inoculated by nipping off the sealed end of the lateral arm, and introducing the inoculating needle through the orifice. The needle deposits the material on the opposite side of the tube: it is then withdrawn and the lateral orifice again sealed. The fluid is then tilted so as to wash down the inoculating matter. The inoculated tube is then placed in an incubator.

The remaining tubes, flasks, and pipettes (see Fig. 17) are all used in the cultivation of microbes. Some are used for storage purposes; while others are used as culture tubes, flasks, etc. A very good storage flask has been recently described by Dr. Sims Woodhead.[1] This flask (Fig. 18) was devised in order to do away with the troublesome method of filling test-tubes, etc., with a pipette. A large flask (containing bouillon) is fitted with an india-rubber stopper with two holes. 'Through these pass two tubes, one with a thistle-head tube running to near the surface of the fluid, *i.e.* about two-thirds of the distance down into the flask, the other passing just through the stopper. To the shorter tube is fitted a piece of india-rubber tubing

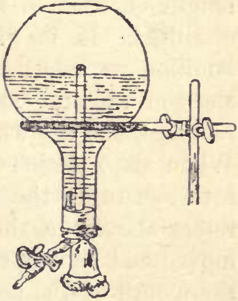

FIG. 18.
WOODHEAD'S STORAGE FLASK.

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[1] *Proceedings of Royal Physical Society of Edinburgh*, vol. ix. p. 537.

on which is a Mohr's clip, and to the other end of this tubing is fitted a piece of glass tubing with a constricted orifice. A plug of carefully sterilised cotton wadding is pushed into the thistle-head, the india-rubber stopper is pushed into the neck of the flask, and then a sheet of cotton wadding is placed over the whole of the tubes and the mouth of the flask, and is held in position by an india-rubber band. The flask is placed in a steam steriliser, where it may be left for a sufficient length of time to allow of it becoming perfectly sterilised. It is filled nearly a third full with bouillon or gelatine, after carefully removing the sheet of wadding and the stopper; these are then replaced, and the whole is again sterilised as usual. When the gelatine or bouillon is to be drawn off into test-tubes, the flask is *inverted* and held in a retort stand, the sheet of wadding is carefully removed and folded, the glass nozzle is inserted into the mouth of the test-tube, the clip is opened and the gelatine or bouillon escapes; all the air passing into the flask, being filtered through the wadding in the thistle-head tube, is thoroughly sterilised. If the whole of the gelatine or bouillon is not withdrawn, all that is necessary is to replace the sheet of wadding (care having been taken to preserve the inner surface, by folding it inwards). There is no necessity to sterilise after this has been once done, all that is necessary subsequently is to heat sufficiently to render the peptonised gelatine fluid; but this is not required if the stock flask contains bouillon. This apparatus is specially useful

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for milk, as the cream always rises to the surface and is so left to the last.'

In all the vessels previously mentioned the nutrient fluid is sterilised by heat; but in certain cases it is necessary to sterilise the fluid without the application of heat: this is performed by means of the apparatus devised by M. Chamberland, of the Pasteur Institute. The fluid is forced by a hand-pump through porous porcelain; and by this means it is sterilised.

In addition to the apparatus, etc., already mentioned in this chapter, a well-fitted laboratory should contain: gas-burners with mica chimneys, water-baths, hot-water filters, platinum needles, wire cages for test-tubes, test-tube stands, glass damp chambers, graduated cylinders, glass dishes and capsules, thermometers, syringes, meat press, 'glass benches,' desiccators, anatomical jars, iron box for glass plates, mouse cages, beakers, glass rods, glass and india-rubber tubing, chemical balance and weights, as well as the various nutrient materials, stains, hardening, imbedding, and mounting materials, and chemical reagents. The last are useful for the extraction and analysis of ptomaïnes and similar bodies.

Although we have detailed the most important pieces of apparatus for the bacteriological laboratory, there are others of importance, but as these are only used for special purposes they will be described later in the volume, *i.e.* in their proper places.

It only remains for us to say on this subject that all the apparatus, etc., used by the English, French, and German schools of bacteriologists may be obtained from Messrs. F. E. Becker & Co., 33 Hatton Wall, Hatton Garden, London.

CHAPTER III

THE METHODS OF CULTIVATING, STAINING, AND MOUNTING MICROBES, ETC.

Cultivation Media.—There are two forms of media used in the cultivation of microbes—one fluid and the other solid. The fluid media were first used by the French school, while the latter (*i.e.* the solid media) were originated by Dr. R. Koch and his followers. Both fluid and solid media have their own special advantages, and both are now used in every bacteriological laboratory.

Of the *fluid media,* the first to be described is bouillon (beef, pork, or chicken broth). This medium is prepared in the following manner:—one pound of lean beef (pork or chicken) is minced by passing it through an ordinary mincing or sausage machine. The minced beef is thoroughly mixed with 1000 cc. of distilled water, and the mixture allowed to stand for twenty-four hours. It is again thoroughly mixed, and then boiled for about an hour. As the fluid is always more or less acid, it is necessary to render it neutral or slightly alkaline, this being done by the addition of a solution of pure sodium carbonate. The point at which the fluid becomes neutral or slightly alkaline is easily ascertained by the ordinary test-papers (litmus and turmeric). It is essential to neutralise any acids, because they are well known to interfere with the growth of many microbes. The extract so obtained is strained through fine linen, and finally filtered through Swedish filter paper. If the filtrate is still acid, add a little more sodium carbonate solution; remove the fat by skimming; add distilled water to make up to the original bulk; and again filter (by means of a hot-water filter, Fig. 19) into a large storage flask or into sterilised test-tubes provided with sterilised plugs of cotton-wool. These vessels and their contents are then heated in the steam steriliser for half-an-hour on each of three successive days.

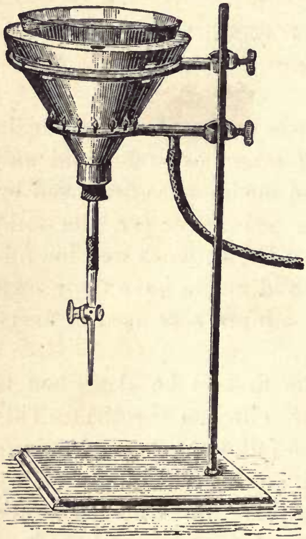

FIG. 19. HOT-WATER FILTER.

Sometimes the beef extract or bouillon is modified by the addition of other materials. Dr. P. Miquel adds common salt in such proportions as to make a 0·5 per cent. solution. MM. Roux and Nocard add glycerine to the bouillon before it is finally sterilised. This glycerine-bouillon is an excellent medium for the growth of *Bacillus tuberculosis*.

Liebig's extract (5 to 1000) and Cibil's extract of beef (20 to 1000) may also be used for the same purposes as bouillon; but these extracts require very careful sterilisation by Professor Tyndall's method of discontinuous heating.

Liquid blood serum is used in drop-cultivations, etc. It is obtained by collecting the blood of a healthy sheep, calf, or horse, in sterilised flasks or glass cylinders with stoppers. The vessel or vessels containing the blood are placed in an ice-box or in ice-cold water for about twenty-four hours, when the separation of the clot will be completed. The fluid serum is then transferred, by sterilised pipettes (see Fig. 17 G), into sterilised test-tubes provided with cotton-wool plugs. The test-tubes and their contents are then heated in a serum steriliser for an hour or two at 60° C. on six successive days. Up to this point the serum forms a fluid medium; but in the majority of cases blood serum is used as a *solid* medium. To solidify it, the serum (contained in test-tubes, watch-glasses, or capsules) is placed in an inspissator, kept at a temperature between 65° and 68° C., until solidification takes place.

Milk is also used as a fluid medium. It is best sterilised at 120° C. in a steam digester or an autoclave. By this means it is readily sterilised in about fifteen minutes. Milk can also be sterilised in the steam steriliser at 100° C., but it is necessary to heat it for an hour on the first day, and for thirty minutes on each of the following two days, that is (unless an autoclave is used), milk must be sterilised by discontinuous heating.

Various infusions of hay, wheat, cucumber, and turnip, and decoctions of malt, prunes, raisins, and horse-dung are used as cultivation media. They are sterilised by being heated in the steam steriliser for thirty minutes on three or four successive days. The mucors grow well in decoctions of malt and horse-dung; various *Aspergilli* in a decoction of malt and prune-juice; and an infusion of hay is a useful medium for the growth of *Bacillus subtilis.*

Urine and other fluids of the body are used as cultivation media: these are sterilised after the manner described for bouillon.

Besides the above-mentioned *fluid* media, there are two others which are useful for the growth of certain microbes and moulds. One of these is Pasteur's fluid, which contains 10 parts of pure cane-sugar, 1 part of ammonium tartrate, the ash of 1 part of yeast, and 100 parts of distilled water. The other is known as the Cohn-Mayer fluid, which contains in 100 cc. of distilled water half a gramme each of magnesium sulphate and potassium phosphate, one gramme of ammonium tartrate, and 0·5 gramme of tricalcium phosphate. Pasteur's and Cohn-Mayer's fluids are sterilised by the method of discontinuous heating; or if they are placed in sealed flasks and sterilised in an autoclave, the sterilisation is complete in about fifteen minutes. Both of these fluids are useful media for the cultivations of the various species of *Torulæ* or yeasts. Test-tubes, flasks, etc., are filled with fluid media by means of sterilised pipettes; or, better still, the fluid media can be run directly into the cultivation vessels by using Woodhead's storage flasks, which have already been described. For the inoculation of various media, pieces of platinum wire, either mounted in sealed glass-tubing, or unmounted, are used. They are sterilised by being heated in the Bunsen flame. To inoculate any medium, the sterilised needle, or a capillary pipette (Klein), is first dipped into the inoculating substance, and then transferred to the medium. Where Aitken's tubes and similar devices are used the medium contained therein is inoculated by using unmounted sterilised needles. These are dipped into the inoculating substance and then dropped into the fluid medium. To inoculate

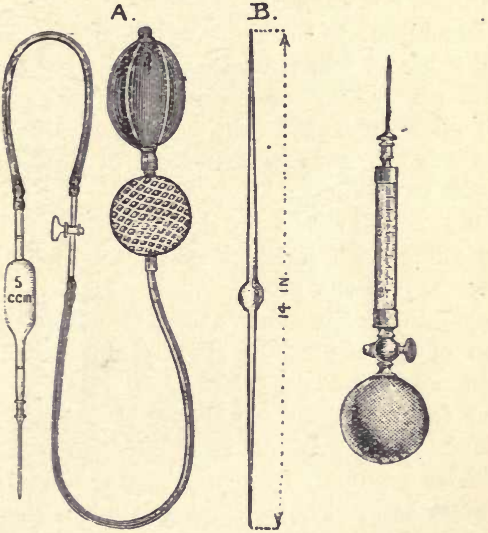

Fig. 20. Injection Syringes.
(A, Dr. Pétri's. B, Dr. Klein's. C, Dr. Koch's.)

animals, platinum needles or injection syringes (Fig. 20) are used; but in every case these instruments must be thoroughly sterilised before use. As Pétri's and Koch's syringes cannot be heated without destruction, they are sterilised by being immersed in a solution of mercuric chloride or mercuric iodide; and after this these syringes should be washed with sterilised hot water.

In addition to the above, 'glass needles are especially useful when anaërobic microbes are being dealt with, as the smooth surface of the glass does not allow of oxygen (air) being carried down with it along the track, which closes up as soon as the needle is withdrawn.'

We now proceed to describe the *solid media* beginning with nutrient gelatine. This is made according to the process already described for the preparation of bouillon, except that after the filtration of the neutral or slightly alkaline fluid, 100 grammes of the best gelatine,[1] 10 grammes of peptone (albumin), and 5 grammes of common salt are added. The gelatine is allowed to soften and dissolve gradually by gently heating the mixture in a water bath. The nutrient gelatine is then sterilised as usual, and filtered into tubes or flasks where it solidifies. The tubes and flasks being filled with the nutrient gelatine must be sterilised in a steam steriliser for a quarter of an hour on three successive days. If these tubes show no signs of turbidity after about a week's incubation, they may be considered sterile.

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[1] Coignet's gold label gelatine is the best for this purpose.

Solid egg albumin is sometimes used as a cultivation medium. The white of an egg is poured on to a slab of glass (sterilised), where it is coagulated and sterilised by being heated in the steam steriliser, or in the hot-air steriliser if the temperature be properly regulated. Solid egg albumin (Fig. 21) is readily inoculated by means of a platinum needle containing the inoculating material. *Micrococcus chlorinus* grows very well on this medium.

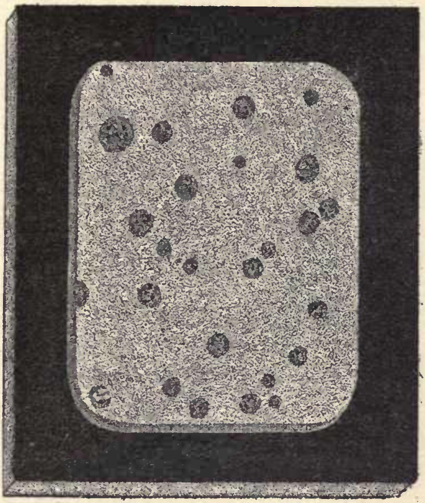

FIG. 21. MICROCOCCUS CHLORINUS.
Growing on sterilised white of egg, after a fourth attenuation.
(The white of egg coagulated and sterilised upon a slab of blackened glass.)

Dr. F. Hueppe's method of cultivating on egg albumin is different from the above. It is as follows:—The shell is first disinfected with a solution of mercuric chloride; a hole is chipped at one end of the egg, and the membrane cut through with a pair of sterilised scissors. The exposed egg albumin is inoculated by means of a platinum or glass needle. The opening is covered with a piece of sterilised paper or cotton wool, which is then painted over and sealed with surgical collodion. The egg is then placed in an incubator.

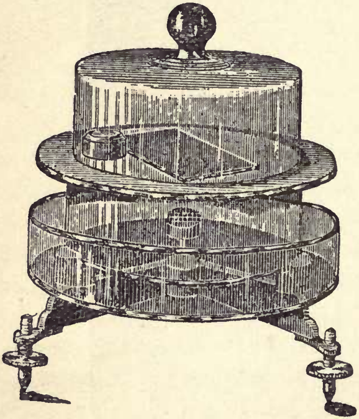

FIG. 22. DAMP CHAMBER.
(For plate-cultivation, etc.)

Cooked potatoes are also used for cultivation purposes. The potatoes (smooth-skinned) are scrubbed and the so-called eyes removed by a sharp knife. They are now soaked for twenty minutes in a solution of mercuric chloride (1 in 1000); washed in water, and then cooked in a steam steriliser for thirty minutes. After cooling, the potatoes are cut by a knife previously sterilised in the naked flame, or in Israël's box placed in a hot-air steriliser. The potatoes are cut[1] through the middle, and the two halves of each potato are then placed in previously sterilised damp chambers (Fig. 22).

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[1] The hands during this operation should have been previously dipped into a solution of mercuric chloride.

The potatoes are inoculated by means of a platinum needle or scalpel containing the inoculating material which is streaked over the surfaces of the potatoes. Second, third, and fourth attenuations may be made from potato-cultivations. Sometimes these cultivations require placing in an incubator, while at other times the growth readily forms at the ordinary temperature of the laboratory. Potatoes form a good medium for the cultivation of numerous microbes, especially the putrefactive and chromogenic forms.

Another solid medium for the cultivation of microbes is agar-agar.[1] This substance is an excellent substitute for nutrient gelatine; for the latter melts at about 26° C., consequently it cannot be used for the cultivation of certain microbes requiring a much higher temperature for their proper growth and development. Agar-agar remains solid up to 50° C. Sterilised nutrient agar-agar is prepared by a similar method to the one already described for preparing nutrient gelatine, with the exception that 20 grammes of agar-agar are used instead of the 100 grammes of gelatine. Although nutrient agar-agar remains solid up to 50° C., it is surpassed in this property by blood serum. Blood serum solidifies at 70° C., and always remains solid. The method for preparing solid blood serum has already been described. Both nutrient agar-agar and solid blood serum are suitable media for the growth of certain microbes requiring a higher temperature than usual.

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[1] **Consists of the dried fragments of certain *Algæ*.**

A good medium for the growth of chromogenic microbes is made of ground rice. The late Dr. Isidor Soyka's formula for the preparation of this medium is as follows:—10 grammes of ground rice, 15 cc. of milk, and 5 cc. of neutral beef bouillon. These ingredients are made into a paste, which is transferred to covered glass dishes or small flasks. The dishes or flasks are then sterilised (in the steam steriliser) for half an hour on three successive days.

Bread-paste is also used as a medium for the cultivation of microbes. It is prepared in the following way:—The crumb of a loaf is broken into small pieces, dried in an oven, and rubbed through a fine sieve. The finely-divided bread is then placed in a sterilised flask, to the depth of half an inch, sterilised water being added until the bread is thoroughly moistened. After replacing the cotton-wool plug the flask (or flasks) is sterilised in the steam steriliser for the same length of time as rice-paste. The flask containing either bread- or rice-paste can be reversed, and is readily inoculated by means of a platinum needle.

To inoculate solid culture media 'the test-tube or flask is held inverted in the left hand, and the plug of cotton wool is twisted once or twice in the mouth of the test-tube to break down any adhesions between it and the neck of the vessel. If the plug is at all dusty, it is well to singe the surface by passing it rapidly through a flame before removing it from its position. The plug is removed and held between two of the unoccupied fingers of the left hand, great care being taken that no part of the plug that passes into the test-tube shall come in contact with any source of infection other than the air itself. At the same time this portion of the plug is directed downwards, in order to avoid any falling germs that may be present in the atmosphere. The platinum or glass needle, with its charge of seed material, is plunged straight into the gelatine mass, then carefully withdrawn and the plug replaced. Where the seed material is also in solid gelatine, the two tubes may be held inverted in the left hand, one between the thumb and finger, the other between the first and second, the plugs being held between the second and third and third and fourth fingers' (Woodhead).

The macroscopical appearances of the test-tube cultivations should always be noted, for many microbes give rise to characteristic growths. Some microbes wholly or partially liquefy the nutrient medium, while others have not this property; but may give rise to pigments, etc., in the medium or media in which they are growing.

Cultivation Methods.—If the original fluid under examination contains different microbes, and it is desired to separate them, so as to obtain pure cultivations of one or all of the microbes present in the original fluid, one of three methods may be used for this purpose. The three methods are known as—plate-cultivations, fractional cultivations, and the dilution method.

In order to utilise the method of plate-cultivation, about three tubes containing sterilised nutrient gelatine or agar-agar are placed in a water-bath heated to 40° C. or 55° C. respectively, so as to melt the medium in each tube. The tubes are then carefully inoculated with a mere trace of the original fluid. The cotton-wool plugs are replaced, and the tubes rolled about so as to distribute the microbes throughout the media. The contents of the tubes are quickly poured into the lower portion of the same number of Dr. Pétri's double dishes (Fig. 23 B) or glass plates (Fig. 23 A). The dishes or plates (which should have been previously sterilised) are then placed in a damp chamber (see Fig. 22). The damp chamber, with its contents, are removed to an incubator, and remain there for several days at about 23° C., or higher if agar-agar is used (*i.e.* according to the temperature required for the growth of the microbes).

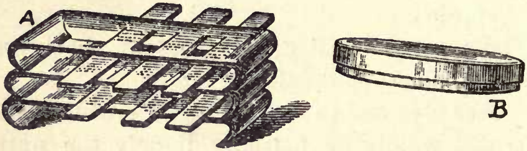

FIG. 23. APPARATUS FOR PLATE-CULTIVATION.
A, Glass Bench with Plates. B, Pétri's Double Dish.

In a few days or so each species will have started a separate growth or colony in different parts of the solidified plate of nutrient gelatine, or agar-agar. The individual colonies are recognisable according to certain macroscopical appearances, such as colour, shape, liquefaction or non-liquefaction of the medium, and the size of the colonies. By plate-cultivation the different species of microbes (*i.e.* in a microbian mixture) separate themselves from each other; and from these colonies pure cultivations of each microbe may be obtained by carefully reinoculating a number of tubes containing sterilised nutrient gelatine or agar-agar. Plates of gelatine or agar-agar (Fig. 24) may also be reinoculated in a similar manner. First, second, and third attenuations may be obtained by this mode of cultivation.

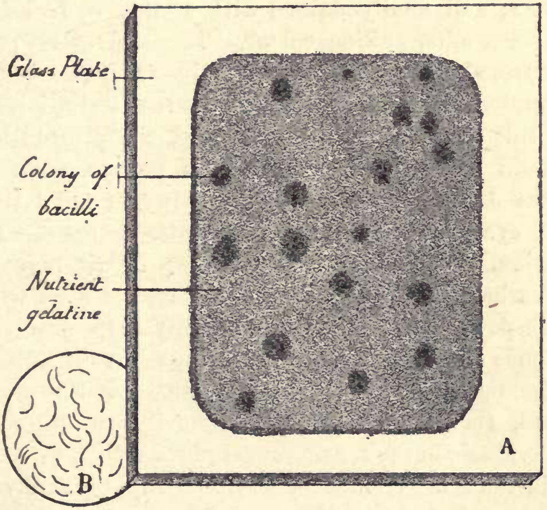

Fig. 24. A Plate-Cultivation of Spirillum Tyrogenum.
A, Colonies growing on nutrient gelatine (sterilised).
B, The spirillum × 1265.

Both the macroscopical and microscopical appearances should be noted. To examine the growth under low power one of the plates should be placed upon the stage of the microscope, and the appearances carefully observed under Zeiss' B, C, and D objectives, or any similar low powers. After this a cover-glass preparation should be made by rubbing a needle, previously dipped into the growth on the plate, on a clean cover-glass. A drop of sterilised water is now added; the cover-glass is allowed to dry; then passed three times through the Bunsen flame; and finally stained with a drop of fuchsine or some other aniline colour. The cover-glass preparation should be temporarily or permanently mounted, according to the methods described later in this chapter. After mounting, the preparation should be examined under high powers, such as Zeiss' J and $\frac{1}{12}$. It should be borne in mind that the eye has to be trained in order to see objects distinctly with such high powers; but, it may be remarked, that 'in all extremely delicate work with high-power lenses, the first difficulty is the greatest. If once an object has been seen, however difficult, it is immensely easier to see it again. On the other hand, there is as great a diversity in different individuals in the sensitiveness of the retina, as there is in the sensitiveness of the olfactory, or auditory nerves. It is impossible to enable some persons to see objects beyond a certain limit of minuteness; as it is to enable others to detect certain scents, or hear notes pitched higher or lower than a given point.'

The *fractional cultivation* method consists in the attempt to isolate, by successive cultivations, the different organisms that have been growing previously in the same culture. A number of tubes containing various cultivation media (sterilised) are inoculated with a mere trace of the original microbian mixture, and are then placed in an incubator for a couple of days or so. It will then be noticed that the different species of microbes (sown in each tube) will not have increased equally in numbers in all the tubes (due, of course, to the nature of the medium, the temperature, and the period of incubation). It is possible that only one species will have developed, so far, in each tube. With these tubes a similar number of tubes are re-inoculated, and so on. By this fractional method of cultivation pure growths are ultimately obtained. For further information concerning this method the reader is referred to Dr. Kleb's paper in the *Archiv für Exper. Pathologie*, 1873.

The *dilution method* consists in greatly diluting a drop of the original microbian mixture with some sterile saline solution (0·5 °/$_0$). A series of tubes, containing different cultivation media (sterilised), are each inoculated, by means of a platinum needle or glass pipette, with a mere trace of the diluted mixture. After about thirty hours' incubation, growths (most likely of one species only), make their appearance in some of the tubes. The original microbian mixture or fluid may be diluted a thousand- or even a million-fold, if the original fluid teems with different microbes. The dilution method has been largely used by Dr. P. Miquel in his examinations of the different waters in and around Paris.

By the fractional, dilution, and plate methods, cultures containing many different species of microbes are capable of being separated one from another. Sometimes a combination of the fractional and dilution methods is used for the same purpose.

The methods of cultivating anaërobic microbes are somewhat different from the above; as the air must be excluded from the cultivation apparatus. In the cultivation of *Bacillus choleræ Asiaticæ,* Koch made use of plate cultivations on which very thin sheets of glass or mica were placed before the gelatine was perfectly set. By this means the colonies of microbes grow out of contact with the air.

A second method for excluding air (*i.e.* oxygen) is to allow the microbes to grow under the receiver of an air-pump which has been exhausted of air.

A third method is to allow the microbes to grow in an atmosphere of carbonic anhydride or hydrogen gas. Another method consists in inoculating a cultivation tube with the anaërobic microbe, and then covering the surface of the medium with a layer of sterilised oil.

Dr. Roux has also devised two methods for this object. One of these methods is to fill a sterilised pipette with sterilised nutrient gelatine. Both ends of the pipette are hermetically sealed. To inoculate the gelatine, one end of the pipette is nipped off, the inoculating material introduced, by a fine glass needle, into the gelatine, and finally the open end of the pipette is again sealed. By this device the microbes grow anaërobically. The second method of Dr. Roux is to boil a quantity of agar-agar in a test-tube; and after quickly cooling, the medium is inoculated with the anaërobic microbe. A layer of melted nutrient gelatine is now poured on the surface of the agar-agar, and when it is cooled a drop of a bouillon cultivation of *Bacillus subtilis* is run on to the surface from a capillary pipette. The tube is then sealed, or the cotton-wool plug is rendered impervious by being luted with warm paraffin-wax. The object of growing *Bacillus subtilis* is that it uses up the oxygen at the surface; consequently the microbe below receives none, or, in other words, it is able to grow anaërobically. To obtain inoculating material from Roux's tube, it is broken at the bottom and a sterilised needle inserted into the lower growth.

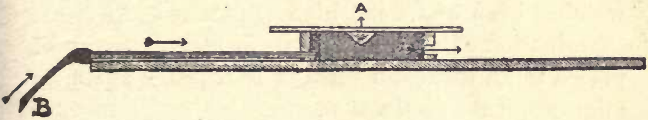

FIG. 25. DROP-CULTURE CELL.
(With arrangement for admitting Gases into the Cell.)

In place of the *Bacillus subtilis*, the layer of nutrient gelatine is covered with a solution containing one part of pyrogallic acid to ten parts of a solution of potassium hydroxide (10 per cent.). The potash solution of pyrogallic acid may be replaced, with advantage, by a 3 per cent. solution of ferrous sulphate, or a 2 per cent. solution of cuprous chloride; both of these compounds (the author has found) prevent the entrance of air.

A *drop culture* forms a useful method for studying the growth and multiplication of microbes under low or high power objectives. For this purpose a glass cell is required. This is made by cementing a sterilised glass ring ($\frac{3}{4}$ in. diam. × $\frac{1}{8}$ in. high) to a microscopic slide, which has been thoroughly cleaned and sterilised (Fig. 25). The upper edge of the ring is moistened with olive oil or vaseline; and the cell is covered over by means of a thin cover-glass, previously sterilised by passing it through a Bunsen flame. The surface of the sterilised cover-glass (A) contains a drop of bouillon or other medium, along with the microbes for examination. A drop or two of sterilised water should be deposited at the bottom of the cell; *i.e.* upon the upper surface of the glass slide. This arrangement forms a miniature damp chamber, in which the growth of microbes may be watched even under the highest powers. After the examination of the cell and its contents, it may be placed in an incubator until it is required again for microscopical examination.

To study the action of *heat* on drop cultures, the warm stages of Schäfer, Ranvier, Israël, Schultze, Stricker, etc., are often used upon the fixed stage of the microscope.[1] The action of various *gases* on drop-cultures may be watched by a modification of the glass cell as represented in Fig. 25. The gases enter through B. The author has used this device during his researches on the action of certain gases on *Bacillus tuberculosis.* The action of the voltaic current or discharges of faradaic electricity may be observed by simple modifications of the drop-culture cell.

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[1] An excellent piece of glass apparatus is used by the Rev. W. H. Dallinger, F.R.S., for ascertaining the thermal death point of microbes. (See *Proc. Roy. Soc.*, 1878.)

Drop-cultures form ready means for studying the complete life-history of any microbe.

The methods for examining *fluids* and *fresh tissues* are as follows: (1) blood, urine, saliva, pus, tears, culture-fluids, and other liquids containing microbes, are easily examined microscopically by placing a drop of the liquid on a glass slide and covering it with a thin cover-glass; (2) when microbes for examination are growing on plates of nutrient gelatine, a small portion of the culture should be taken up on a sterilised platinum or glass needle and placed in a drop of sterilised water on a glass slide. After thinning, the preparation is covered with a cover-glass and examined under low and high powers; (3) for the microscopical examination of fresh tissues, they should be teased out with sterilised needles in dilute glycerine or salt solution (sterilised), then temporarily mounted, in either liquid, on a glass slide and covered with a thin cover-glass. If there is an excess of glycerine or salt solution round the edges of the cover-glass, it must be removed by placing small pieces of filter or blotting-paper in contact, which will soon absorb the superfluous fluid, but the paper must not be left too long or it will drain the fluid from under the cover-glass. In the examination for micrococci and other small microbes, the tissues should be first treated with acetic acid, and then with a solution of potassium hydroxide (potash), the object being to dissolve and disintegrate fatty and albuminous globules which might be mistaken for microbes. Alcohol and ether are also useful agents for dissolving small globules of fat.

It has been recorded that a certain foreign medical professor mistook minute globules of fat for so many micrococci; and certainly the illustrations in his paper indicated that such was really the case. Therefore, let all bacteriologists, young and old, be very sceptical at times as to what they think they see with the highest powers of the microscope. Without wishing to detract an iota from the honesty of purpose and truth of our fellow-workers, we are sure that a good deal unintentionally has been said to have been seen with the microscope which has never been seen at all. We set to work longing to discover something newer than the last new thing. We hope to find it, we begin to think we have found it, and we may go so far as to make ourselves believe we really did see it once. The event must be recorded; we proclaim it, and in so doing propagate error. Therefore, let it be borne in mind that to use the highest powers with accuracy requires continual practice; even when the retina of the eye is sensitive enough to appreciate light-waves proceeding from such organisms as the smallest micrococci.

Staining Cover-glass Preparations and Tissues.—To prepare a cover-glass preparation for staining, a sterilised cover-glass is smeared with the microbian matter (solid or liquid), or with blood, pus, etc., by means of a sterilised needle or capillary pipette. The excess of material is squeezed out by means of an additional cover-glass placed over the original one. The two glasses are then separated, each bearing a small portion of the microbian matter. After drying for a few minutes, they are passed rapidly (three or four times) through a Bunsen flame. To stain the preparations they are allowed to float (with the prepared side downwards) on the surface of an aqueous solution of methyl violet, gentian violet, or magenta, for a short time. After this the cover-glasses are washed with water, then spirit, and finally with sterilised distilled water. They are then drained, dried, and mounted in Canada balsam or any suitable medium. The preparation must now be set aside to dry, and when thoroughly dry it is 'ringed' or sealed with Hollis' glue.

Before continuing the description of the various methods of staining, we describe the preparation of several staining fluids :—(1) Gentian violet stain is prepared by rubbing 2 grammes of gentian violet in a glass mortar with 10 cc. of alcohol (sp. gr., 0·83), in which has been dissolved 2 cc. of aniline oil. To this is added 90 cc. of distilled water; (2) Koch's methyl violet stain contains the following ingredients :—Aniline water, 100 cc.; an alcoholic solution of methyl violet, 11 cc.; and absolute alcohol, 10 cc.; (3) the stain known as Bismarck brown is prepared by dissolving 2 grammes of Bismarck brown in 15 cc. of alcohol, and then adding 85 cc. of distilled water; (4) hæmatoxylin solution contains 2 grammes of hæmatoxylin, 2 grammes of alum, and 100 cc. each of alcohol, glycerine, and distilled water; (5) the methylene blue stain is prepared by dissolving 2 grammes of methylene blue in the same quantities of alcohol and water as are required to prepare the Bismarck brown stain;

(6) Ranvier's picro-carmine stain contains 1 gramme of carmine, 3 cc. of ammonia, 10 cc. of distilled water, and 200 cc. of a cold, saturated solution of picric acid; (7) vesuvin stains are prepared by dissolving 3, 4, or 5 grammes of vesuvin in 100 cc. of distilled water; (8) Dr. Gibbes' solution for double staining contains 2 grammes of magenta and 1 gramme of methyl violet, which are triturated in a glass mortar with 15 cc. of alcohol (in which has been dissolved 3 cc. of aniline oil). To this mixture is added 15 cc. of distilled water; (9) Gram's iodine solution is prepared by dissolving 1 gramme of iodine and 2 grammes of potassium iodide in 300 grammes of distilled water; (10) Löffler's stain contains 30 cc. of a concentrated alcoholic solution of methylene blue, and 100 cc. of an aqueous solution of potassium hydroxide (1 in 10,000); (11) an eosin solution is prepared by dissolving 5 grammes of eosin in 100 cc. of distilled water.

We now continue the description for staining microbes and tissues. To stain tissues containing microbes, place them in either an aqueous solution of methyl violet (2·25 grammes in 100 cc. of water), or one of gentian violet (containing the same strength of solution), and allow them to remain in the solution for some hours. When deeply stained, wash in water to remove the excess of the stain, and then lay them out flat in methylated spirit, and let them remain until no more colour comes away. Transfer them to absolute alcohol, and then oil of cloves, and mount in Canada balsam (Gibbes).

To double stain bacilli which produce spores, the cover-glass preparation should be floated for half an hour on the surface of a small quantity of hot magenta and aniline stain.[1] The magenta is discharged from the bacilli by washing in water, in alcohol, or weak nitric acid, according to the species. The preparations are then treated (for three or four minutes) in a solution of methylene blue, and finally washed with water, drained, dried, and mounted in Canada balsam or other mounting media. By this method the spores are stained red, while the bacilli are blue.

Koch's method for staining tubercle bacilli is as follows:—Cover-glass preparations of the sputum, etc., are placed in a solution containing 1 part of a concentrated solution of methylene blue, 2 parts of a potash solution (10 per cent.), and 200 parts of distilled water. The preparations remain in the solution (heated to 40° C.) for twenty-four minutes. They are then washed in water, and placed in an aqueous solution of vesuvin for two or three minutes; again washed, and subsequently treated with alcohol, oil of cloves, and finally mounted in Canada balsam. Koch's method stains the bacilli blue, and the nuclei, etc., brown. ‘All the other forms of bacteria which Koch has as yet examined in this way are stained brown, with the exception of the bacilli found in leprosy, which also retain the methylene blue in preference to the vesuvin. These bacilli

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[1] This stain is prepared by mixing together 5 cc. of aniline oil and 100 cc. of distilled water. The mixture is filtered, and to the filtrate is added a concentrated alcoholic solution of fuchsine or magenta, until a precipitate begins to be formed.

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may also be stained by other aniline dyes if the solution be made alkaline by the addition of caustic potash or soda.'

To stain the flagella of certain microbes, Koch recommends that the cover-glass preparations should be floated on a concentrated aqueous solution of hæmatoxylin. They are then transferred to Müller's fluid,[1] or to a five per cent. solution of chromic acid. By using either of these reagents the flagella are stained a brownish-black colour.

On the other hand, Dr. Dallinger[2] does not think that Koch's method of staining brings out the flagella well. Dallinger uses high powers and the microbes alive.

Dr. Crookshank has, however, succeeded in photographing the flagella by staining with a concentrated alcoholic solution of gentian violet. The preparation is then rinsed in water, dried, and mounted in Canada balsam.

Gram's method for staining microbes in tissues is as follows:—The sections containing the microbes are soaked in absolute alcohol for twelve minutes, and then placed in a gentian-violet-and-aniline solution[3] for about three minutes. The sections are then placed in a solution of iodine (in potassium iodide) for several minutes, or until they are of a brown colour. After this they are transferred to absolute alcohol until decolourised; they are then placed in oil of cloves, and finally mounted in Canada balsam. As Gram's method only gives a faint colour to the tissues, they may be stained a deeper colour by immersing the sections (after decolourising with alcohol) in an aqueous solution of vesuvin, eosin, or Ranvier's picro-carminate of ammonia. They are finally washed in alcohol, and mounted as already described.

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[1] This fluid contains 2 grammes of potassium bichromate, 1 gramme of sodium sulphate, and 100 cc. of distilled water.

[2] *Journal of Royal Microscopical Society*, 1878, p. 172.

[3] This is similar to Koch's methyl-violet-and-aniline stain, except the methyl violet is replaced by gentian violet.

One of the best methods for staining cover-glass preparations is the one devised by Ehrlich. The cover-glass preparations are made to float (with the prepared face downwards) in a solution of fuchsine made in the following manner: 5 cc. of aniline oil and 100 cc. of distilled water are mixed together and filtered. To the filtrate is added a concentrated alcoholic solution of fuchsine. The preparations remain in this solution for fifteen minutes; they are then washed in nitric acid (one part of nitric acid to two parts distilled water) and rinsed in distilled water. An after-stain of methylene blue or vesuvin gives the nuclei, etc., a blue or brown colour, while the tubercle-bacilli or other pathogenic microbes are stained red. The elegance of this method is that the tubercle-bacilli impregnated with fuchsine resist the action of nitric acid, whilst the saprophytic microbes (present in sputum and saliva), nuclei, etc., are immediately decolourised by the acid. Both Ehrlich's and Koch's methods are also applicable for staining tubercular and other tissues.

The Ehrlich-Weigert is another method for staining microbes *in situ*. The tissues are placed in a warm solution of aniline-methyl-violet,[1] and then decolourised with nitric acid (one in two). The tissues may be stained brown by immersing them in an aqueous solution of Bismarck brown or vesuvin. In this case the microbes are blue and the tissues brown. Other aniline colours may be used, but the decolouriser is nitric acid. The stained sections are washed, cleared in oil of cloves, and then mounted in Canada balsam.

In the Baumgarten method cover-glass preparations of sputum are placed in a very dilute solution of potassium hydroxide (potash), and after being slightly pressed on the microscopic slides they are ready for examination. By this method the bacilli (tubercle) are seen in the unstained condition. This is a quick method of examining phthisical sputum, as it does not take more than ten minutes.

Gibbes' rapid double-staining method is applicable for staining sections as well as cover-glass preparations. No decolourising agent is used, while the double-staining process is performed in one operation. The preparations are allowed to remain in a warm aniline-magenta-methyl-violet solution for five minutes, or in the case of sections for several hours. They are washed in methylated spirit until no more colour comes away. The preparations are now dehydrated in absolute alcohol, dried and mounted in Canada balsam dissolved in xylol. By this method the tubercle-bacilli and certain other pathogenic microbes are stained red, while the putrefactive bacteria and micrococci are blue. This method is a rapid one, and is, consequently, recommended for the busy medical man.

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[1] The above solution is prepared by mixing together 100 cc. of a saturated aqueous solution of aniline and 11 cc. of a saturated alcoholic solution of methyl violet. The filtered mixture is the Ehrlich-Weigert stain.

The Ziehl-Neelsen method of staining the tubercle-bacilli is a modification of the Ehrlich-Weigert method already described. The cover-glass preparations or sections are stained in the following dye: 1 gramme of fuchsine is dissolved in 10 cc. of absolute alcohol, and to this is added 100 cc. of an aqueous solution of carbolic acid (5 per cent.). The mixture is then heated. In the hot dye sections are stained in six or seven minutes; and cover-glass preparations are stained in about three minutes. The preparations or sections are now placed for a second or so in 90 per cent. alcohol, then in dilute sulphuric acid (25 per cent.), when the pink colour is replaced by a yellowish brown. The preparations, etc., are then transferred to a solution of lithium carbonate. They are afterwards stained in an aqueous solution of methylene blue, cleared in oil of cloves, and mounted in Canada balsam. This method (also known as the carbol-fuchsine method) gives excellent results.

To ascertain the presence of tubercle-bacilli in tuberculous milk, the best plan is to pass the milk through one of the ordinary centrifugal machines used in the dairy; and then to take the sediment (after the separation of the cream and skim milk) for examination. In lieu of a centrifugal machine, the milk should be allowed to stand for about twenty-four hours in a chemical separator (Fig. 26) surrounded with ice. The sediment (containing the bacilli) is drawn off from the separator by means of a tap (see Fig. 26); and a few drops of the sediment are dried on a cover-glass, and examined in the ordinary way.

Dr. W. Kühne's methylene blue method is one of the best means of staining for general purposes. It is prepared by dissolving 1·5 grammes of methylene blue in 10 cc. of absolute alcohol; and 100 cc. of an aqueous solution of carbolic acid (5 per cent.) are added. Preparations are stained in this dye from five minutes to two hours; and sections remain in it for twenty-four hours. They are washed in water, followed by acidulated water,[1] and are then transferred to a solution of lithium carbonate (5 per cent.). They are again washed in water, dehydrated in absolute alcohol, placed in aniline oil, and transferred to terebene for two or three minutes. After this treatment the preparations are washed in xylol, and finally mounted in Canada balsam. This stain is useful for the bacilli of leprosy, glanders, tuberculosis, and almost any microbe.

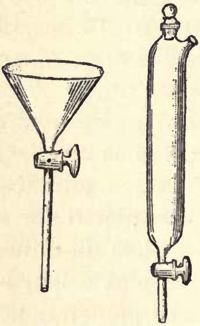

Fig. 26.
Chemical Separators.

Cover-glass preparations of anthracic blood, etc., are floated on a hot alcoholic solution of fuchsine for thirty minutes. They are then decolourised in weak hydrochloric acid, and after-stained with methylene blue. By this means the spores are stained red and the bacilli blue.

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[1] Two or three drops of hydrochloric acid to 100 cc. of distilled water.

Anthrax-bacilli and spores may also be stained with an aqueous solution of gentian violet, fuchsine, or any of the aniline dyes; if the cover-glass preparation is first passed ten or eleven times through the Bunsen flame.

Sections of anthracic tissues are well stained by Gram's method, and after-stained with picro-carminate of ammonia, or eosin.

The bacillus of glanders is stained by the method of Schütz. The sections are placed in an alcoholic potash solution of methylene blue[1] for twenty-four hours. They are then washed in acidulated water,[2] transferred for five minutes to 50 per cent. alcohol, ten minutes to absolute alcohol, clarified in oil of cloves, and finally mounted in Canada balsam. As already stated, the bacillus of glanders (*Bacillus mallei*) may be stained by Kühne's methylene blue method.

There are three principal methods for staining the bacillus of syphilis. (1) Lustgarten's method consists in placing the sections of syphilitic tissues, etc., for about twenty-four hours in a solution containing 100 cc. of aniline-water (5 per cent.) and 11 cc. of a saturated alcoholic solution of gentian violet. They are now heated for two hours at 60° C. After this treatment, the sections are placed for three or four minutes in absolute alcohol, transferred to a solution of potassium permanganate (1·5 per cent.) for ten minutes, and decolourised by immersion in concentrated sulphurous acid. The sections are then dehydrated in absolute alcohol, clarified in oil of cloves and mounted in Canada balsam. (2) The next method is that of Doutrelepont and Schütz. The sections of syphilitic tissues containing the bacilli are immersed in an aqueous solution of gentian violet (1 per cent.), and are after-stained with an aqueous solution of safranin (1 per cent.). (3) The last method is that of De Giacomi, in which the preparations are immersed in a hot solution of fuchsine containing a drop or two of ferric chloride. They are then decolourised in a concentrated solution of ferric chloride, and after-stained with Bismarck brown or vesuvin. In both the Doutrelepont-Schütz and De Giacomi methods, the preparations (after staining) are dehydrated, clarified, and mounted in the usual way.

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[1] This stain contains equal parts of a concentrated alcoholic methylene blue solution and a solution of potash (1 in 10,000).

[2] Water containing 5 per cent. of acetic acid.

Sections of tissues containing the *Bacillus lepræ* are stained by immersion in a solution of fuchsine in aniline-water. They are then decolourised in hydrochloric acid (33 per cent.), and after-stained with methylene blue. Another method is, first to tie a piece of thread around the base of one of the leprosy nodules, so as to cut off the blood supply; then with a fine-pointed scalpel (see Fig. 5) a small puncture is made, when a clear fluid exudes. From this fluid, cover-glass preparations are made. Cover-glass preparations and sections of leprosy tissues may be stained by the methods of Ehrlich, Ziehl-Neelsen, and Gram.

The method (devised by Dr. Löffler) for staining the *Bacillus diphtheriæ* consists in placing the sections in Löffler's alkaline methylene blue (already described) for about five minutes. The excess of stain is removed by very dilute acetic acid (0·5 per cent.). They are then dehydrated in alcohol, clarified in cedar oil, and mounted in Canada balsam. Sections may also be stained by Gram's method; and Dr. Klein has produced beautiful stained sections of diphtheritic membranes[1] by staining them with rubin[2] and methyl blue. By this method the bacilli are stained blue, while the nuclei and necrotic substances of the membranes are stained red.

To stain the *Bacillus typhosus* (the microbe of typhoid fever), there are several methods in use. For tissue-staining, the method of Gram may be used. Some bacteriologists recommend steeping the sections for twenty-four hours in methylene blue; but this stain possesses the disadvantage of quickly fading. The colour, however, may be fixed by placing the sections either in a solution of picro-carminate of ammonia, or of iodine dissolved in potassium iodide, or in ammonium picrate. Dr. Kühne's method consists in allowing the sections to remain for some time in a concentrated aqueous solution of oxalic acid, washing them in water, and afterwards staining with methyl blue dissolved in a solution of ammonium carbonate (1 per cent.). To demonstrate the spores of this bacillus, cover-glass preparations and tissue-sections must be placed in a *hot* solution of fuchsine. They are then decolourised with nitric acid (see Ehrlich's method), after-stained with methylene blue, and mounted as usual, after dehydration and clarification in the media already described.

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[1] See *Report of Medical Officer of the Local Government Board*, 1889-90, p. 143.

[2] Rubin is rosaniline nitrate.

The most important methods for staining the *Micrococcus pneumoniæ* are as follows:—(1) By the method of Gram. (2) Cover-glass preparations of pneumonic sputum and exudations are treated with acetic acid, stained with gentian violet, and temporarily mounted in distilled water, or water and glycerine, *i.e.* for immediate examination; or they may be dried and permanently mounted in Canada balsam.

Cover-glass preparations of gonorrhœal pus, blood, or of artificial cultivations of the *Micrococcus gonorrhœæ* are readily stained with an aqueous solution of fuchsine. This method may be also used for demonstrating the presence of the same micrococcus in the tears of new-born infants suffering from purulent ophthalmia of gonorrhœal origin.

The cholera bacillus (*Bacillus choleræ Asiaticæ*) is stained by the following methods: (1.) The discharges, etc., containing the microbe are spread and dried on a cover-glass. They are then stained with an aqueous solution of fuchsine, washed with water, dried, and mounted in Canada balsam. (2.) The hardened sections[1] of the intestines are placed for twenty-four hours in a strong aqueous solution of methylene blue; and finally treated in the usual way. (3.) 'The best method yet described of demonstrating the cholera bacillus in the discharges of the intestines is that recommended by Cornil and Babès, who spread out one of the small white mucous fragments on a microscopic slide, and then allow it to dry partially; a small quantity of an exceedingly weak solution of methyl violet in distilled water is then flowed over it, and it is flattened out by pressing down on it a cover-glass, over which is placed a fragment of filter paper, which absorbs any excess of fluid at the margin of the cover-glass. Cholera bacilli so prepared and examined with an oil-immersion lens (Zeiss' $\frac{1}{12}$ homog., Oc. 3 or 4) may then be seen; their characters are the more readily made out because of the slight stain they take up, and because they still retain their power of vigorous movement, which would be entirely lost if the specimen were dried, stained, and mounted in the ordinary fashion.'

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[1] Hardened in absolute alcohol.

For staining cover-glass preparations of the blood of patients suffering from relapsing fever (*i.e.* containing the *Spirillum Obermeieri*), fuchsine, gentian violet, and Bismarck brown have been used with considerable success. Sections of the brain, liver, lungs, kidneys, etc., of monkeys or human beings dead of the disease, are best stained with Bismarck brown, vesuvin, or chrysoidine.

Cover-glass preparations of the blood, exudations of the throat, etc., from cases of scarlatina (*i.e.* containing the *Micrococcus scarlatinæ*) are stained with a saturated solution of methyl violet. The micrococci, adhering to the scales of the desquamating epidermis in such cases, are also stained with the same dye. As the scarlatina micrococcus has been found in diseased cow's milk,[1] such milk should be treated by the method described for the examination of tuberculous milk (see p. 75).

Bacillus butyricus is best stained with a solution of iodine in potassium iodide.

Actinomyces is usually stained by Plaut's method. Sections of nodules, tumours, etc. (from cases of Actinomycosis) are immersed for ten or twelve minutes in a stain containing two grammes of magenta, 3 cc. of aniline oil, 20 cc. of alcohol (sp. gr. 0·83), and 20 cc. of distilled water. The stain (with the sections) is warmed to 45° C. The sections are rinsed in water, and after-stained in a strong alcoholic solution of picric acid for about eight minutes. They are then immersed in water for five minutes, in alcohol (50 per cent.) for fifteen minutes; and finally passed through absolute alcohol and oil of cloves, and mounted in Canada balsam. The tissues containing this fungus may be examined in the fresh state. A little of the tissue, etc., is transferred to a microscopic slide, teased out with needles, and then temporarily mounted in a drop of glycerine and water.

We have given most of the principal methods for the examination of microbes. It may be remarked that nearly all microbes can be stained with the various aniline dyes; although their capacity for absorbing these dyes differs considerably. This capacity or affinity for aniline dyes is of great use to the bacteriologist to ascertain the presence of microbes, and to differentiate in many instances morphological details which in the unstained condition are not discernible.

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[1] See Dr. Klein's *Reports to the Local Government Board*, 1885-8.

Hardening, Imbedding, Cutting, and Mounting Preparations.—Many medical men and students on reading the different staining, hardening, imbedding, cutting, and mounting processes ' which any tissue has to undergo before it can be examined with the microscope, will be inclined to think it very tedious work. It is, however, a mere matter of routine, and when once this routine is established, the whole thing is comparatively simple. It takes very little time to change the hardening fluid, and if the student gets into the habit of looking over the bottles on the shelf every morning where he keeps tissues in the process of hardening, a glance at the labels will show those requiring a change. When the sections are mounted and examined under the microscope, he will find himself amply repaid for all his trouble if he has faithfully carried out the different processes in every detail. It is always better to have one or two shelves devoted to those preparations which require changing; and those which require fresh fluid often, as for instance those hardening in chromic acid should be kept by themselves. Each bottle should be labelled, and the tissue, date, and hardening fluid clearly written on the label. Every morning this shelf should be examined, and the hardening solution changed in those requiring it, the date being each time written on the label, so that it may be seen at a glance how long the tissue has been in the fluid, and whether the hardening agent ought to be renewed. Müller's fluid and bichromate of potash preparations may be placed by themselves, and need only be looked at occasionally.'

The best *hardening* agents are absolute alcohol, methylated spirit, Müller's fluid, chromic acid solution, potassium bichromate solution, and osmic acid. (1.) Pieces of an organ, etc., should be cut from ½ in. to 1 in. cubes, and placed in one of the hardening solutions. If absolute alcohol or methylated spirit is used the tissues should remain in the spirit from two to three days. Many delicate tissues, however, cannot be placed in strong spirit without shrinking; to obviate this such tissues are first placed in dilute spirit (one part of water to two parts of spirit). In this mixture the tissues remain about twenty-four hours and are then transferred to the strong spirit for one or two days. After this they are ready for imbedding and cutting. (2.) Müller's fluid is an excellent hardening agent. To prepare it, dissolve two parts of potassium bichromate, one part of sodium sulphate, and 100 parts of distilled water. The preparations to be hardened should remain in the fluid from two to three weeks. When the fluid becomes cloudy it requires changing; but it retains its hardening properties for a long time. The preparations, after being hardened in Müller's fluid, should be washed in water, and then placed in dilute spirit (one of water to two of spirit) for about twenty-four hours. Sometimes the treatment with dilute spirit is dispensed with, especially if the sections are to be cut immediately. (3.) Chromic acid solution is really a mixture of chromic acid and spirit. It is prepared by dissolving one gramme of chromic acid in 600 cc. of distilled water. Two parts of this solution is then mixed with one part of methylated spirit. The material to be hardened is placed in this fluid for twenty-four hours; the fluid is then changed, and again every third day; the material being hardened in from eight to twelve days. The material should not be allowed to become brittle, which it does if it remains too long in this fluid. After hardening the material is washed in water, and the sections cut immediately (*i.e.* after imbedding), or it is placed in dilute spirit for twenty-four hours, and then transferred to strong methylated spirit. In this fluid the material may remain for an indefinite time; that is, if it is not required for immediate use. (4.) A two per cent. solution of potassium bichromate is sometimes used, especially where tissues require slow hardening. 'This solution takes from three to seven weeks to harden, according to the size of the specimen, and the frequency with which the solution is changed.' (5.) A 0·5 per cent. solution of osmic acid is used for hardening certain preparations—such as the internal ear. This solution must be protected from light; for this purpose the bottle in which it is kept should be painted externally with black oil paint.

To decalcify small bones or teeth, they are placed in Ebner's or Kleinberg's solution. Ebner's solution contains five grammes of sodium chloride (salt), 5 cc. of hydrochloric acid, 20 cc. of distilled water and 100 cc. of alcohol. Kleinberg's solution is made as follows: 100 cc. of a saturated aqueous solution of picric acid are added to 2 cc. of strong sulphuric acid. The mixture is filtered and 300 cc. of distilled water are added. In either solution the materials (to be decalcified) remain until sufficiently softened; they are then allowed to soak in water, and finally passed through weak spirit to absolute alcohol.

For cutting sections either by hand or by the microtome, it is necessary (as a rule) to imbed the material in one of the imbedding mixtures. If the material to be cut has been preserved in alcohol, it is better first soaked in water for about ten hours to remove the spirit, and then placed in mucilage[1] for about five hours. For cutting with the non-freezing microtomes, the material is imbedded in celloidin or paraffin, mounted on cork.[2] To imbed in celloidin the hardened material is first placed in a mixture of alcohol and ether for thirty or forty minutes; then transferred to a solution of celloidin (dissolved in equal parts of alcohol and ether) from two to twenty hours.[3] A cork placed in the clamp of the microtome is smeared on its upper surface with a solution of celloidin, which is left to harden. When the material is ready, it is mounted upon a prepared cork (*i.e.* it is placed on the smeared surface); and a little celloidin solution is poured over the material so as to cover it. The mounted material is now placed in 70 per cent. alcohol in order to harden the celloidin (which has a pasty consistence). In a few hours or so the imbedded material will be ready for cutting with one of the microtomes already described. Schanze's microtome is a useful instrument for cutting sections of materials imbedded in celloidin. In cutting a tissue imbedded in celloidin or mounted directly on cork, the razor and tissue should be kept wet with alcohol, and the sections carefully transferred to alcohol. The sections (if from celloidin material) are placed in oil of cloves in order to dissolve out the infiltrated celloidin. They are then ready for staining, etc.

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[1] Mucilage is prepared by making a solution of gum Acacia.

[2] If the material is firm enough it is sometimes mounted on cork without being imbedded.

[3] The length of time depends on the nature of the material. It is longer for spongy structures like the lungs.

For fixing pieces of *firm* materials directly on corks either glycerine-gelatine[1] or gelatine is used. These substances are liquefied by the application of heat.

Paraffin wax for use as an imbedding material is first dissolved in chloroform, and then used in a similar manner to the solution of celloidin already described. The imbedded material must be cut perfectly dry, and the sections removed to xylol.

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[1] Glycerine-gelatine is prepared as follows: to 10 parts of gelatine add sufficient water to allow the gelatine to swell up; pour off the water, and melt the gelatine. To the melted gelatine add 10 parts of glycerine, and finally a few drops of some germicidal agent, preferably carbolic acid. The latter is added in order to preserve the glycerine-gelatine.

The xylol dissolves out the infiltrated paraffin, and the sections are then placed in alcohol to extract the xylol. After this treatment they are ready for the staining process.

Instead of celloidin and paraffin, wax-and-oil mixture [1] and vaseline-and-paraffin mixture are used for imbedding purposes.

Before alcohol-hardened tissues are cut with the *freezing* microtomes they must be soaked in water for ten minutes, this process to be followed by five hours' soakage in mucilage. After this they are frozen and cut with the microtome, whose razor must be perfectly sharp and free from notches. Tissues hardened in Müller's fluid (if they have not been subsequently placed in alcohol) are at once dried with blotting-paper, then frozen, and finally cut. Fresh tissues are covered with mucilage, frozen, and cut. The razor should be moistened with a solution of gum, and the sections transferred with a camel-hair brush to warm distilled water for fifteen minutes—the object being to dissolve out the mucilage. They are then ready for staining, etc., with the exception of sections of fresh tissues, which should be placed, before staining, in a 0·6 per cent. saline solution, so as to prevent too much shrinking of the sections.

In cutting sections with the microtome 'very little force is required in pushing the razor or knife through the material, and if it is sharp a very slight turn of the screw each time will enable one to cut a section, which ought to be so thin as to be almost invisible.'

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[1] Equal parts (by weight) of white wax and olive oil are melted together.

After staining, etc., the sections are mounted in various media on glass slides (3 in. × 1 in.), and covered with thin cover-glasses.[1] 'When high-power lenses are to be used it facilitates the work very much to know the exact thickness of the cover-glass under which the specimen is mounted, and with very high powers, or those with wide angles of aperture, the cover-glass must be at least 0·004 in. to enable the lens to work through it.' All Zeiss' objectives in fixed mounts are corrected for a cover-glass of medium thickness (between 0·15 and 0·2 mm., or 0·006 and 0·008 in.). In the higher series from CC upwards the thickness of the cover-glass consistent with the most perfect correction is indicated on the side of the mount by small figures (mm.). As a rule, it is sufficient for ordinary work to use cover-glasses of an estimated medium thickness.

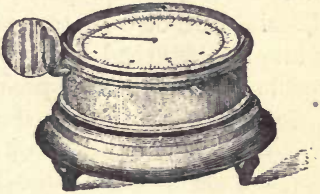

FIG. 27. ZEISS' COVER-GLASS TESTER.

Oil-immersion objectives are within wide limits independent of the thickness of the cover-glass. But considerable variations in the thickness of the cover-glass may be compensated for—by slightly lengthening the body-tube for thinner cover-glasses; and by slightly shortening the body-tube of the microscope for thicker cover-glasses. Zeiss makes a good tester (Fig. 27) suitable for the exact measurement of the thickness of cover-glasses. The measurement is effected by a clip projecting from a box; the reading is given by an indicator moving over a divided circle on the lid of the box. The divisions show hundredths of a millimetre, and the instrument is capable of measuring up to five millimetres.

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[1] The round ones are better than those that are square.

Before use the glass slides and cover-glasses should be perfectly clean.

Many methods for permanently mounting tissues and cover-glass preparations have already been described. For fresh tissues glycerine is often used, while for hardened tissues the following mounting media have each their special advantages:—

(*a*) Canada balsam dissolved in xylol.
(*b*) Canada balsam dissolved in benzol.
(*c*) Canada balsam dissolved in chloroform and turpentine.
(*d*) Dammar varnish.

After the tissues have been stained, they pass through the following processes:—Washing off the excess of stain, dehydrating, clearing or extracting the infiltrated material used in the imbedding process, etc.; mounting, cementing, or sealing; and finally, labelling the slides. The following list gives the various agents for the above-mentioned processes:—

(1) Washing agents, . . .	Water. Dilute spirit. Absolute alcohol.
(2) Dehydrating agent, . .	**Absolute alcohol.**

(3) Clearing agents, . . .	Oil of cloves. Oil of cedar. Xylol. Aniline oil. Terebene.
(4) Mounting agents, . . .	Canada balsam. Dammar varnish. Glycerine.
(5) Cementing agents, . .	Hollis' glue. Cements (various). Gold size. Black asphalte varnish.

To mount a *fresh* specimen, the section should be placed with the utmost care in the centre of a glass slide. The section should not be folded in any part, therefore it must be carefully spread out with needles. This must be performed without stretching the specimen. After this has been done, wipe off all moisture with a clean cloth. Now take up 'a cover-glass and place a drop of glycerine in the centre, invert and place it horizontally on the preparation, leaving the weight of the cover-glass to spread out the glycerine.' If there is an excess of glycerine round the edges of the cover-glass, it must be carefully absorbed by filter or blotting-paper, but on no account should the cover-glass be removed. To seal, ring, or cement the preparation, paint round the edge of the cover-glass and a little way on the slide, a ring of Hollis' glue or Dammar varnish. Hollis' glue[1] is better than Dammar varnish, for it is not acted upon by the cedar oil used with oil-immersion lenses. The sealing of microscopic preparations with Hollis' glue or any other cementing agent is performed with a camel-hair brush and a turn-table (Fig. 28). The slide is fixed with the clips of the turn-table, the table revolved, and the brush containing the cement is held in a vertical position, so as to touch the edge of the cover-glass. By this means a ring of the cement is deposited, which dries in a day or two. The preparation is permanently sealed, and should now be labelled and placed in the cabinet.

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[1] Gold size is sometimes used for sealing glycerine preparations.

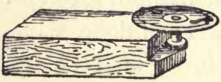

FIG. 28. TURN-TABLE.

The various preparations of Canada balsam and Dammar varnish are prepared as follows:—(*a*) To prepare xylol balsam it is necessary to dissolve Canada balsam in xylol until it has the consistency of treacle; (*b*) benzol balsam is prepared by first drying the Canada balsam until it is brittle. It is then dissolved in benzol until it has the same consistency as the xylol balsam. If these mounting fluids get thick on keeping, they are thinned by the addition of xylol and benzol respectively; (*c*) chloroform-turpentine balsam is prepared by dissolving 3 ozs. of Canada balsam in 1 oz. of chloroform and 1 oz. of turpentine. If this medium gets thick, it is thinned by the addition of chloroform; (*d*) Dammar varnish is prepared by first dissolving 1½ oz. of powdered gum Dammar in 1½ oz. of turpentine, and filtering. At this point ½ oz. of gum mastic is dissolved in 2 ozs. of chloroform, and the solution filtered. The two solutions are finally mixed together, and again filtered.

These fluids are used for mounting *hardened* tissues, and they should be preserved in stoppered or well-corked bottles; while for daily use a small drop-bottle of each fluid should be placed on a table set apart for mounting purposes. It may be mentioned that xylol balsam is the best mounting fluid for *stained microbes*; chloroform-turpentine balsam acts well with *hardened sections*; and benzol balsam is the most useful solution for *general* microscopic purposes.

These mounting fluids are all used in the same manner, therefore a description of mounting in xylol balsam will also apply to the other fluids.

The sections having been stained and washed, they are placed for twelve minutes in absolute alcohol contained in a watch-glass: the alcohol dehydrates them. They are now drained, and then placed in oil of cloves to clarify them. While in this medium they should be carefully straightened out with needles.[1] Having now placed a drop of xylol balsam in the centre of the slide, it is spread out with a needle; then a section is carefully lifted[2] out of the oil of cloves, drained, and placed in the xylol balsam. A small drop of xylol balsam is placed on the under surface of a clean cover-glass, which is lowered on to the section. With practice and perseverance, slight pressure with the forefinger is all that is required to produce a slide devoid of air-bubbles. To remove these bubbles small air-pumps have been devised, but they are not to be recommended; 'the only thing to be done when an air-bubble lodges in a cavity of the section, and refuses to move in any way by gentle pressure, is to lift the cover-glass, and transfer the section to oil of cloves, and then remount it.' As the mounting of sections may be performed in the summer, the xylol balsam is much thinner than usual (due to the heat), and therefore takes a much longer time to set. In such cases a mounting clip (Fig. 29) is useful to keep the cover-glass from moving, *i.e.* until the balsam sets. After this the slide should be sealed with Hollis' glue, or some other cementing agent, as already described.[1]

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[1] Ordinary steel needles mounted in wooden handles.

[2] A lifter is made by beating out one end of a copper wire, and then turning up the broad portion. Lifters made of German silver may be purchased at Messrs. F. E. Becker & Co., of Hatton Garden, London.

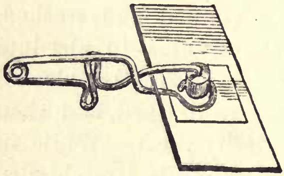

FIG. 29. MOUNTING CLIP.

Methods of Introducing Microbes into Living Animals.—In such experiments guinea-pigs, rabbits, mice, fowls, etc., are used. Pure cultivations of microbes and infectious matter are introduced into the animal body by the following methods:—

(*a*) Inhalation.
(*b*) Swallowing.
(*c*) Direct inoculation.
(*d*) Special operations.

(*a*) An animal is made to inhale the infectious matter, etc., disseminated by means of a spray; (*b*) the infectious matter is mixed with the animal's food; (*c*) the infectious matter is introduced into the animal body by cutaneous or subcutaneous inoculation or injection; (*d*) by the fourth method mentioned above (*i.e.* special operations), the infectious matter may be injected into the duodenum, or introduced into 'the peritoneal cavity by the performance of abdominal section.' These and other operations are used as means of introducing microbian matter into the living animal. But it cannot be too firmly impressed upon the mind that all operations should be performed with antiseptic precautions; and the instruments, as well as the hands of the operator, should be thoroughly disinfected.

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[1] For further information see Martin's *Manual of Microscopic Mounting.*

Before closing the present chapter we give a few remarks on what is known as *the unit of microscopical measurement.* It has been the general practice among bacteriologists to give the dimensions of microbes in terms of a thousandth part of a millimetre, which is called a micro-millimetre, and is known by the symbol μ.[1] This unit is of great importance, for 'it is always easier to conceive the size of any object, and especially to realise the comparative sizes of two objects, when their dimensions are given in terms of a unit smaller than either; for instance, it is difficult exactly to comprehend the length represented by $\frac{1}{289}$ of an inch, and few people can readily compare such dimensions as $\frac{1}{15}$ and $\frac{1}{20}$ of an inch. This difficulty vanishes when the dimensions are expressed as multiples of a small, properly chosen unit, and not as fractions of a large one. For this purpose a fraction of an inch might be adopted instead of a fraction of a millimetre (mm.); but, at any rate, in measuring the spores of fungi, $\frac{1}{10000}$ of an inch is too large a unit, and $\frac{1}{100000}$ of an inch would be inconveniently small. It happens that, if we take $\frac{1}{1000}$ of a millimetre as our unit, we can express the size of the spores of all fungi, etc., in the fewest possible figures. For instance, many micrococci measure about 1 μ, the spores of *Penicillium* about 3 μ, the spores of many *Myxomycetes* about 10 μ, and so on. If we compare these figures with the following: 0·001 mm., 0·003 mm., 0·01 mm.; or, still more, with these: 0·00004 in., 0·00012 in., 0·0004 in.—we see the great saving effected in the trouble of writing down the dimensions, quite apart from the greater readiness with which they can be compared with one another. But perhaps the difficulty with some is that of realising and actually applying this unit; we will therefore give an easy method by which the size of the micromillimetre may be obtained. Place the microscope in such a position that the image projected upon a piece of white paper is magnified 254 times: this can easily be done by a quarter-inch objective, with the use of the draw-tube, or by placing the paper at a greater distance than ten inches from the eye-piece. Let this position be marked, so that the microscope can be placed in it again at any time. Now copy on the paper, from a scale, an inch divided into ten parts, and with a fine pen subdivide each tenth into five equal parts. Then the value of each of these subdivisions will be 2 μ, and of the whole tenth of an inch, 10 μ. If this scale be carefully copied on a piece of thin cardboard or other suitable substance, the dimensions of any microbe, etc., drawn by the camera lucida or otherwise on the paper in that position of the instrument, can be easily read off in μs. With the aid of a deeper eyepiece or higher objective we can magnify the image 508 times, and then each small division of the scale will represent 1 μ.'

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[1] $1\ \mu = 0{\cdot}001$ mm. $= \frac{1}{25400}$ in., or 0·0000393 in.

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CHAPTER IV

THE ORIGIN, CLASSIFICATION, AND IDENTIFICATION OF MICROBES

SCIENTISTS and non-scientists are agreed that there was a lifeless period in the history of the earth—therefore that life had a beginning. But *when*, *where*, and *how* did life begin? 'As to the *time*, there is no evidence whatever. Life is enormously older than any record of it. Even the higher forms were developed long before the periods in which we first find their remains. As to the *place*, probably in the polar regions, as Buffon suggested in his *Epoques de la Nature*. The earth being a cooling globe, those regions would be the earliest to reach a temperature under which life is possible.' During the past twenty years or so, Buffon's theory has been supported by Comte de Saporta[1] and others; and it is highly probable that in the earliest zoic epochs (especially the north polar) regions of the earth were of a hot and humid nature. Moisture and heat are essential to life; therefore life had its beginnings in water.[2] It is probable that lowly plants (possibly microbes) were the first organised beings which made their appearance on the earth, for it is well known that all microbes require moisture, while many live in water or similar media. From these and other facts it is probable that the *Schizomycetes* were the forms of life which originated in the polar regions of the earth—the other parts of the earth, at that remote time, being too hot for life to exist. But if life originated in the particular part of the earth indicated, this does not explain the origin of life. How did life begin? This question has occupied the thoughts of men in all ages, but if we regard living and non-living matter as composed of elements which are common to both kinds of matter, wherein lies the difference which gives as one result non-living matter, and as another result living matter? The difference must lie in the mixing of these elements. If the first form of living matter were a microbe it originated either by a creative act or by spontaneous generation. Both the theory of creation and that of spontaneous generation account for the origin of life: in fact, the beginning of life can only be explained theoretically, for there is no practical or direct proof of how life originated. On this point Professor Huxley[1] says: 'If it were given me to look beyond the abyss of geologically recorded time to the still more remote period when the earth was passing through physical and chemical conditions, which it can no more see again than a man can recall his infancy, I should expect to be a witness of the evolution of living protoplasm from not living matter. I should expect to see it appear under forms of great simplicity, endowed, like existing fungi, with the power of determining the formation of new protoplasm from such matters as ammonium carbonates, oxalates, and tartrates, alkaline and earthy phosphates, and water without the aid of light. That is the expectation to which analogical reasoning leads me; but I beg you to recollect that I have no right to call my opinion anything but an act of philosophical faith.'

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[1] *L'Ancienne Végétation Polaire.*

[2] See Professor Moseley in *Nature*, September 3, 1885.

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[1] *Critiques and Addresses*, p. 238.

Besides the two great theories which account for the origin of life from mineral matter,[1] there are others, which we now describe. It has already been stated that putrefaction is the result of life, not of death—the result of microbian activities[2]—but formerly many naturalists believed that by putrefaction the organic elements which had composed the body of the dead animal formed themselves by free creative power into independent beings, which differed entirely from those from which their material was produced, yet are in every case animated, and have the power of propagation; thus the albumin and fat globules take the form of microbes, perhaps also of yeasts and moulds, or even of those little infusorial animals, whose presence never fails in corruption. This mode of origin has been called equivocal generation or *generatio æquivoca.*[3] Other naturalists dispute the possibility of living beings, however small and simple, ever originating in any other way than from germinal matter which sprang from the same form of life; and they insist that the belief in the equivocal origin of microbes is that last remnant of an old superstition, which the light of science has not entirely banished. In ancient times it was thought that serpents and frogs originated from slime, that caterpillars were generated from decayed leaves, vermin from filth, and worms from spoiled meat. Now-a-days every child knows that all these things are fables; every housewife knows by experience that no maggots originate in meat if the blow-fly is prevented by a wire-screen from entering and depositing its eggs. They have learned, through careful covering, to keep away the minute mould-spores, which settle with other dust from the air, and which colonise on their preserved fruits; they know that trichina and tapeworm only originate from raw or half-cooked pork, in which these animals were already present in the embryonic stage. Even the farmer no longer believes that the grain rust (*Puccinia graminis*) originates from chilling, but that it springs from spores which are scattered by the barberry bushes (*Berberis vulgaris*), or other fallen stalks, and that the blight may be prevented in corn crops, if the seed (before sowing) is steeped in a solution of iron sulphate or copper sulphate, in order to kill the spores which cling to it.[1]

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[1] Those of creation and spontaneous generation.

[2] The microbes being introduced from the air, water, etc.

[3] The equivocal origin of microbes must be distinguished from the spontaneous generation, which we have already alluded to; for in the latter case there existed no organisms on the earth.

Concerning microbes and their related 'fermentations,' the above-mentioned observations lead without doubt to the conclusion that they do not originate through equivocal generation; for when nitrogenous material from the animal or vegetal world is heated in flasks, even at as low a temperature as 70° C., all the microbes are killed, and if the entrance of new germs from outside is in every way prevented, and it were possible to keep the flasks for ever, no microbes would ever originate of themselves. On the contrary, the entrance of a single germ, in each flask, is sufficient to cause multiplication, and with it putrefaction. If microbes originate from putrid matter through equivocal generation, putrefaction must appear before the microbes; but experience shows the contrary, that putrefaction is a consequence of the development and growth of microbes. Within the last few years a theory has been advanced to account for the origin of microbes, which has caused some sensation, viz., that under certain conditions the ordinary mould-fungus will give rise to moving germs of extraordinary minuteness; such germs are capable of developing into microbes, into yeasts, and finally again into the mould-fungus. When microbes are found in the blood or organs in certain diseases, the authors of this theory of *pleomorphism* are satisfied that the spores of the common mould germinate in the human body; that these spores first swarm as microbes, but under suitable culture may be nourished into different species of moulds. However, unprejudiced research has not given the slightest proof that microbes stand in any connection with the development of yeasts, moulds, or other fungi. They always originate, as far as we know at present, from spores, etc., of the same kind (Cohn).

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[1] See Dr. Griffiths' book, *The Diseases of Crops*, pp. 128-132 (Bell & Sons).

Concerning the doctrine of pleomorphism, it may be stated that Lankester,[1] Van Tieghem, Zopf, Cienkowski, Billroth, Neelsen, Hauser, and others have noticed that certain microbes pass through various phases during their life-histories. And Sattler, Gravitz, and Büchner, believe that they have transformed certain non-pathogenic microbes into pathogenic forms by simply cultivating the former in different media or under different physical conditions. For instance: Sattler[2] states that he has transformed the non-pathogenic *Bacillus subtilis* into a pathogenic form, capable of producing infectious ophthalmia, by cultivating the microbes (at 35° C.) in an infusion of jequirity seeds. Gravitz believed that he had transformed the non-pathogenic moulds—*Aspergillus glaucus, Penicillium glaucum*—into pathogenic forms by cultivating them in alkaline media at about 40° C. Büchner states that he has transformed *Bacillus subtilis* into *Bacillus anthracis* and *vice versâ*: 'that by successive cultivation of *Bacillus anthracis* under constant variation of the nutritive material, he saw it assume the morphological and physiological characters of *Bacillus subtilis*.'

Klein, Koch, Cohn and others do not accept the theory of pleomorphism, or the transformation of microbes; and Klein[3] has proved most conclusively that no pathogenic microbe is ever transformed into a non-pathogenic form, or *vice versâ*. In fact, he says that 'those organisms which are connected with morbid processes possess this pathogenic power *ab initio*; and are not due to any peculiar condition of growth.' If a harmless microbe could be proved capable of transformation into a harmful form, 'the whole doctrine of the infectious diseases is involved in such a case; for if in one case it can be unmistakably proved that a harmless microbe can be transformed into a pathogenic organism, *i.e.* into a specific virus of an infectious disease, and if this again can, under altered conditions, resume its harmless property, then we should at once be relieved of searching for the initial cause in the outbreak of an epidemic. But in that case we should be forced to contemplate, as floating in the air, in the water, in the soil, everywhere, millions of microbes which, owing to some peculiar unknown condition, are capable at once to start any kind of infectious disorder, say anthrax (Büchner), infectious ophthalmia (Sattler), and probably a host of other infectious diseases, and thus to form the starting-point of epidemics. And the only redeeming feature, if redeeming it can be called, in this calamity, would be the thought that the particular microbe would by-and-by, owing to some accidental new conditions, again become harmless' (Klein).

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[1] *Quarterly Journal of Microscopical Science*, 1873, p. 408.

[2] *Wiener Medic. Wochenschrift*, 1883.

[3] *Micro-Organisms and Disease*, pp. 207-231 (3d ed.).

The transformation of microbes into different forms is entirely opposed to the Darwinian law. To one who has fully comprehended the meaning and the operation of this law, it will be at once apparent that there must be error somewhere in the matter. 'If the law of actual variation,' says Dr. Dallinger, 'with all that is involved in the survival of the fittest, could be so *readily* brought into complete operation, and yield so pronounced a result, where would be the stability of the organic world? Nothing would be at one stay. There could be no permanence in anything living. The philosophy of modern biology is that the most complex forms of living creatures have derived their splendid complexity and adaptations from the *slow* and majestically progressive variation and survival from the simpler and the simplest forms. If, then, the simplest forms of the present and the past were not governed by accurate and unchanging laws of life, how did the rigid certainties that manifestly and admittedly govern the more complex and the most complex come into play? If our modern philosophy of biology be, as we know it is, true, then it must be very strong evidence indeed that would lead us to conclude that the laws seen to be universal break down and cease accurately to operate, where the objects become microscopic, and our knowledge of them is by no means full, exhaustive, and clear. Moreover, looked at in the abstract, it is a little difficult to conceive why there should be more uncertainty about the life-processes of a group of lowly living things, than there should be about the behaviour, in reaction, of a given group of molecules. The triumph of modern knowledge is a knowledge —which nothing can shake—that Nature's processes are immutable. The stability of her processes, the precision of her action, and the universality of her laws, are the basis of all science, to which biology forms no exception. Once establish, by clear and unmistakable demonstration, the life-history of an organism, and truly some change must have come over Nature as a whole, if that life-history be not the same to-morrow as to-day; and the same to one observer, under the same conditions, as to another.

'But the fact that there is no evidence of any direct relation evolutionary between two such forms as *Bacillus subtilis* and *Bacillus anthracis*, the fact that there is no ready way either naturally or artificially of their being changed into each other, must not blind us to the fact that such an evolutional relation *in the past* is eminently probable, nay almost certain. It may, in all probability must, have taken an indefinite time in the past to effect; but being once effected, the specificity is continued as in every other form by inheritance.'

There are certain conditions under which a microbe may appear to have altered its properties. For instance, Chauveau[1] has shown that *Bacillus anthracis* loses its virulence when submitted to the action of compressed oxygen; but it does not lose its vaccinal property after this treatment. This new character is said to be maintained by suitable cultivation. Although *Bacillus anthracis* may lose its virulence under such abnormal conditions as already alluded to, it does not become a non-pathogenic microbe, for it still preserves one of the most essential attributes that indicate the infectious nature of the pathogenic microbe, viz., its vaccinal property. Besides, Chauveau has further shown that the non-virulent *Bacillus anthracis* may be revivified by degrees when grown in suitable media. These researches do not point to any transformation of *Bacillus anthracis* into a non-pathogenic species, but simply show that oxygen under pressure is capable of modifying the microbe's pathogenic power. In fact, microbes have the power of adapting themselves to considerable variation of external conditions; but this does not involve *permanent* change in the organism.

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[1] *Comptes Rendus de l'Académie des Sciences*, tome 109.

Microbes belong to the vegetal kingdom; in other words, they are fungi. As they multiply by repeated subdivision, and also frequently reproduce themselves by spores, which are formed endogenously, they are grouped together in a class called the *Schizomycetes*, splitting fungi, or Spaltpilze, as the German naturalists term them.

The forms of microbian cells vary considerably—they are round, ovate, elliptical, cylindrical, etc. Microbes live isolated, singly, or in larger or smaller colonies, or, in many cases, united in pairs, or many together in threads or groups. Nearly all microbes possess two different modes of life: one of motion and another of rest. In certain conditions they are extremely motile, and when they swarm in a drop of water or other fluid they move among each other in all directions; sometimes rotating round their longitudinal axis, while in other cases the movement is an oscillating one, or the threads alternately bend and straighten themselves, etc. At other times the motile microbes become motionless. In this state many of them aggregate together and excrete a gelatinous material which entirely envelopes them. This colony is termed a zoogl≈ìa, in fact it is the resting stage of the particular microbes. In the zoogl≈ìan stage, microbes often produce spores.

Microbes multiply by fission (*i.e.* division) and spore-formation. The warmer the air, etc., the faster proceeds the division, and the stronger the multiplication; in a lower temperature it becomes slower, and ceases entirely at the freezing-point of water. Their fecundity is enormous, and would, in a very short time, choke up the earth; but this rapid rate of increase is kept in check by the limited supply of food, climatic conditions, and the struggle for existence.

As an example of the enormous fecundity of microbes we describe the rate of reproduction of a common form, viz., *Bacillus subtilis.* This bacillus attains a certain length and then divides across into two. 'Each half grows to the size of the parent, and then similarly divides, and so on as long as food and other conditions of their life are present. *Bacillus subtilis* has been observed to divide in this way every half-hour, a rate which gives in twenty-four hours more than three hundred billion of individual microbes as the offspring of one parent. They are extremely minute, varying from $\frac{1}{20000}$th of an inch to the $\frac{1}{1000}$th of an inch in length.'

As already stated, microbes propagate by fission and by spore-formation. The following table gives those that are produced only by fission, and those that multiply by fission and spores:—

	Mode of Propagation.	General Remarks.
Micrococci .	Fission . .	Spherical and oval in form.
Bacteria .	Fission . .	Rod-shaped microbes, generally smaller than bacilli, and devoid of spore-formation.
Bacilli . .	Fission and spores	Rod-shaped microbes, many are provided with flagella.
Vibriones .	Fission and spores	Curved or more or less wavy rods provided with flagella.
Spirilla . .	Fission and spores	Spiral-shaped microbes.
Spirochætæ	Fission and spores	Filamentous and wavy microbes.

Concerning the reproduction of the micrococci, if the division takes place in one direction only, the resulting form (if the two cells remain together) is a diplococcus, dumb-bell, or colon (:). The diplococcus may again divide, without separation, forming a streptococcus or chain, which may become curved or even twisted in appearance. Sometimes the division of these microbes is in two directions, resulting in four cocci (::), which is termed a merismopedia, or in three directions forming a sarcinacoccus or sarcina.[1]

All microbes require for their nutrition and growth oxygen, carbon, nitrogen, certain salts, and water. Although some microbes are anaërobic, they require oxygen, which is obtained from the carbohydrates and albuminoids of the medium in which they live, or from the free oxygen which may be dissolved in that medium.

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[1] A division into a large and an indefinite number of cells is termed an ascococcus.

Before considering the various classifications of microbes, we mention the fact that microbes in general are sometimes called Bacteria, but as there is a genus of that name, it is better that the word should be applied only when one is alluding to microbes of that genus. The study of microbes (which includes all forms of *Schizomycetes*) has been consequently termed Bacteriology; but it is an unfortunate name, which, at the present time, cannot well be replaced by another.

Microbes may be simply divided into aërobic[1] and anaërobic[2] forms. *Bacillus spinosus* and *Bacillus œdematis maligni* are examples of the former; while *Micrococcus candicans* and *Bacillus subtilis* are examples of the latter kind.

Microbes may be also divided into pathogenic (disease-producing), septic (putrefactive), zymogenic (fermentive), and chromogenic (pigment-forming) forms.

The *Schizomycetes*, which Sachs includes in his group the *Thallophytes*, have been classified by Cohn[3] into five genera:—

(1) Spherobacteria or micrococci.

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[1] Those requiring free access of oxygen (air).

[2] Those which do not require free oxygen.

[3] *Beiträge zur Biologie der Pflanzen*, 1872 *et seq.*

(2) Microbacteria or bacteria.
(3) Desmobacteria or bacilli and vibriones.
(4) Spirobacteria or spirilla.
(5) Spirochætæ.

This classification is founded upon the idea that all the various morphologically or physiologically distinct forms belong to different species. Koch's researches with plate-cultivations have given great support to the classification of Cohn, which, in our opinion, is the best, that is, from the bacteriologist's point of view. In such a classification a micrococcus produces nothing but a micrococcus, a bacillus nothing but a bacillus, and so on.

Zopf (who is the great apostle of the doctrine of pleomorphism) divides microbes into four groups:[1]—

(1) Coccaceæ.
(2) Bacteriaceæ.
(3) Leptothricheæ.
(4) Cladothricheæ.

The first group contains streptococcus, merismopedia, sarcina, micrococcus, and ascococcus forms; in fact this group only contains cocci. The second group contains the following genera:—*Bacterium*, *Spirillum*, *Vibrio*, *Leuconostoc*, *Bacillus*, and *Clostridium*. Most of these forms, according to Zopf, pass through a coccus stage. The third group contains four genera:—*Crenothrix*, *Beggiatoa*, *Phragmidiothrix*, and *Leptothrix*. This group (like the second) is believed to possess coccus, rod, and thread forms. The fourth and last group only contains the genus

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[1] *Die Spaltpilze*, 1885.

Cladothrix, which shows coccus, rod, thread, and spirillar forms.

Baumgarten divides microbes into two groups, each containing three genera:—

Monomorphic Group.	Pleomorphic Group.
Coccus.	Spirulina.
Bacillus.	Leptothrix.
Spirillum.	Cladothrix.

The genus *Bacterium* is entirely dispensed with in this classification; and Flügge, who modified Cohn's classification, has submerged the genus *Bacterium* into the genus *Bacillus*, as both these forms were rod-shaped; but it should be borne in mind that the bacteria do not produce spores, whereas in the bacilli spore-formation is of common occurrence.

Hueppe's classification is based on the mode of reproduction, or, rather, fructification; and the late Dr. De Bary divided them into two groups: Microbes which produce endospores, and microbes which produce arthrospores. But as we know so little about spore-formation in the *Schizomycetes*, Hueppe's and De Bary's classifications are of very little practical value at the present time.

'The determination of species rests upon the accumulated evidence afforded by a thorough knowledge of their life-history.' The form of the microbe, the physiological, pathological, and other changes it effects, and the microscopical and macroscopical appearances under cultivation, must be collectively taken into account. This determination or identification of species will be considered in the next chapter.

CHAPTER V

THE BIOLOGY OF MICROBES, ETC.

In this chapter we describe nearly all the more important microbes; but the microbes present in such diseases as tuberculosis, cholera, diphtheria, scarlatina, etc., will be described in Chapter VI.

MICROCOCCI.

Micrococcus prodigiosus.—This microbe, which measures from 0·5 to 1 μ in diameter, gives rise to a blood-red pigment when grown on boiled potatoes, white of egg, starch-paste, bread, agar-agar, and other media. Fig. 30 represents the macroscopic and microscopic appearances of this microbe. It grows well on agar-agar, which it liquefies. The pigment, which *M. prodigiosus* gives rise to, is insoluble in water, but soluble in alcohol; and in many of its reactions it resembles certain aniline colours.[1] This pigment is only produced under certain conditions, viz., at a temperature of from 20° to 22° C., and after the gelatine or agar-agar has liquefied. As the temperature rises to blood-heat *M. prodigiosus* loses its power of forming the red pigment; but forms casein, lactic acid, and probably other substances. 'When growing and kept in the depth of a solid nourishing material, *i.e.* removed from the free surface, colonies of this microbe grow as colourless micrococci.' They are always present in the atmosphere, and give rise to the phenomena known as 'bleeding bread,' 'blood-rain,' 'bloody sweat,' etc.

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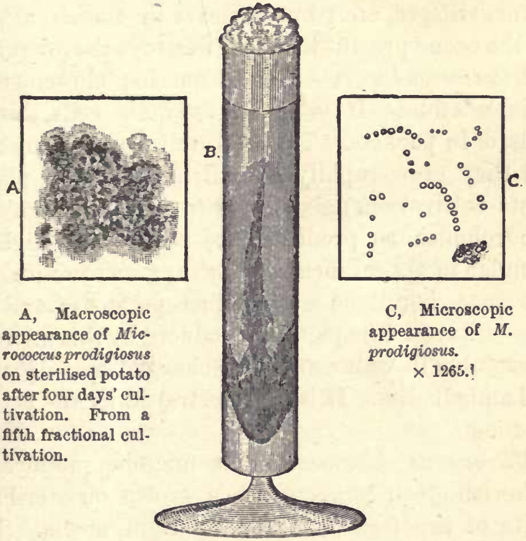
[1] Erdmann in *Journal für Praktische Chemie*, 1866; and Schröter in *Beiträge zur Biologie der Pflänzen*, vol. i. p. 109.



A, Macroscopic appearance of *Micrococcus prodigiosus* on sterilised potato after four days' cultivation. From a fifth fractional cultivation.

C, Microscopic appearance of *M. prodigiosus.* × 1265.[1]

B, A growth of *Micrococcus prodigiosus* in nutrient agar-agar.

FIG. 30. MICROCOCCUS PRODIGIOSUS.

A desiccation of four months at 32° C. (dry heat)[1] does not destroy the vitality of *M. prodigiosus*; but when exposed to the action of ozone the microbe is killed.[2] From these facts one can readily understand how it is that *M. prodigiosus* (as well as other aërial microbes) is always present in the air of towns, villages, etc.; but is never in the air at sea, for the ozone present in sea-air destroys the microbes.

Micrococcus luteus.—This is another chromogenic aërial microbe. It is found as single cells, dumb-bells, or in packets. The cells are 1·2 μ in diameter; and they grow rapidly on nutrient gelatine plates (plate-cultivations) giving rise to a yellow pigment. The colonies, so produced, are round and slightly granular in appearance. *M. luteus* grows in nutrient agar-agar, bouillon; on steamed potatoes; and as drop-cultures. The pigment produced by this microbe is insoluble in water, and is unchanged by sulphuric acid and alkalies. It is also destroyed by the action of ozone.

Micrococcus chlorinus.—This microbe produces a yellowish green pigment when grown on sterilised white of egg (see Fig. 21) and fluid media. The cells are about 1 μ in diameter. The pigment is soluble in water, and is decolourised by acids.

Micrococcus aurantiacus.—The cells of this aërial microbe are 1·5 μ in diameter; and they occur singly, in pairs, or in zooglea. On plate-cultivations they form orange-coloured drops and spots, which ultimately coalesce into equal-sized patches. On fluid media they form an orange-coloured pellicle. *M. aurantiacus* also grows on steamed potatoes and white of egg. The pigment is soluble in water.

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[1] Griffiths in *Proceedings of Royal Society of Edinburgh*, vol. xvii. p. 262.

[2] See Griffiths' *Researches on Micro-Organisms*, p. 184.

Micrococcus fulvus.—Cells 1·5 μ in diameter; they form rusty-red drops and gelatinous masses on horse-dung.

Micrococcus violaceus.—The cells are 1·4 μ in diameter, and occur as bright violet-coloured gelatinous drops or patches on the surface of steamed potatoes exposed to the air.

Micrococcus cyaneus.—The cells are elliptical and grow on potatoes and fluid media, giving rise to a blue pigment when in contact with air. The pigment is soluble in water, and the solution is at first green, but afterwards becomes an intense blue. Acids convert this pigment into a red colouring matter, while alkalies turn it green. There are no characteristic absorption bands shown when a solution of the blue pigment is examined by the spectroscope.

Micrococcus rosaceus.—The cells are from 1 to 1·5 μ in diameter, and give rise to a rose-coloured growth on the surface of nutrient gelatine and agar-agar.

Micrococcus cinnabareus.—This microbe grows very slowly on the surface of gelatine. At the end of eight days the colonies appear as small drops of a red colour; but ultimately the colour becomes red-brown. This microbe occurs in twos, threes, and fours; and very rarely as an isolated coccus. The pigment is soluble in water.

Micrococcus hæmatodes.—This microbe is sometimes found in human sweat. It grows on steamed-egg albumin in a damp chamber placed in the incubator (see Figs. 22 and 14). The pigment produced is a red colour.

Micrococcus flavus tardigradus.—Colonies of this microbe form raised drops of a chrome-yellow colour. In test-tube cultivations, they form small yellow beads along the track of the needle. This microbe does not liquefy the gelatine.

Micrococcus flavus liquefaciens.—The microbe grows in colonies of a yellow colour, and the cells form diplococci and zooglea. They liquefy the gelatine.

Micrococcus versicolor.—Small cocci forming iridescent colonies. The colonies are flat, not raised; and in test-tubes the yellowish colonies have the appearance of small beads, *i.e.* along the needle track. These cocci are found in pairs or in masses.

Micrococcus flavus desidens.—This microbe occurs in the dust of the atmosphere. The cells are 0·8 μ in diameter, and occur singly, as diplococci, and in short chains. They form yellow colonies, which ultimately sink down in the gelatine. The yellow pigment is only formed at the surface of the gelatine, for in the track of the needle the colonies are white.

Micrococcus citreus conglomeratus.—The cells are 1·5 μ in diameter, and occur in the atmosphere and in blennorrhœic pus. On gelatine plates they form citron yellow colonies.

Micrococcus cereus flavus.—The cells are 1·5 μ in diameter, and occur singly, in lemon-yellow groups, or in short chains. They are found in pus.

Micrococcus subflavus.—The cells are 0·8 μ in diameter, and occur singly, in pairs, in tetrads, and zooglœa groups. On gelatine they form white dots, which ultimately become yellow and confluent. This microbe was originally found in vaginal secretions and lochial discharges.

Micrococcus radiatus.—The cells are 0·8 μ in diameter, and occur singly and in short chains. They form 'whitish colonies with a yellowish-green sheen.' The colonies liquefy the gelatine and sink down in it; there developing, in the course of a day or two, a circlet of rays.

Micrococcus pyogenes citreus.—The cells occur singly, in chains, and masses. They grow on nutrient agar-agar and gelatine, giving rise to a lemon-yellow pigment. They are obtained from pus.

Micrococcus pyogenes.—The cells are 1 μ in diameter, and occur in chains or diplococci. They form small colonies which grow slowly; on plate-cultivations they are first white, then pale yellow, and finally become brown. They (the colonies) have no tendency to run together in either plate, stroke, or puncture cultivations, except on agar-agar or blood serum where the mass is thicker in the centre. They do not grow on potatoes; and do not liquefy any medium. They occur in the pus of acute abscesses.

Micrococcus pyogenes aureus.—This coccus occurs in osteomyelitis. It grows on boiled potatoes, nutrient gelatine, agar-agar, and blood serum, giving rise to orange cultures. This microbe liquefies gelatine, and the colonies remain limited to the centre of the liquefying area. *M. pyogenes aureus* is 0·8 to 0·9 μ in diameter, and occurs as diplococci, tetrads, short chains, and in irregular masses. It is fatal in large doses to guinea-pigs, mice, and rabbits if injected into the veins or into the peritoneal cavity. According to Becker, 'when a small quantity of a cultivation was introduced into the jugular vein after previous fracture or contusion of the bones of the leg, the animal died in about ten days, and abscesses were found in and around the bones, and in some cases in the lungs and kidneys.' This microbe peptonises albumin.

Micrococcus ureæ.—The cells are round or oval, and measure 1·25 to 2 μ in diameter. They occur isolated or concatenate or forming a zoogl&oelig;a on the surface of the fluid. *M. ureæ* secretes a ferment which causes the ammoniacal fermentation of urea:

$$CH_4N_2O + 2H_2O = (NH_4)_2\,CO_3.$$

The ferment has been isolated (in aqueous solution), and it is proved that it has the power of converting urea into ammonium carbonate.[1] Besides this well-known microbe, there are certain bacteria, and possibly bacilli, which produce a similar reaction.[2]

Micrococcus pyogenes albus.—The cells are 0·8 to 0·9 μ in diameter, and occur as diplococci, tetrads, short chains, or irregular masses. They grow rapidly on gelatine plates, producing colonies which are white. In test-tube cultivations, a white mass is formed along the needle track. About the third day of growth liquefaction sets in, and ultimately a white deposit settles at the bottom of the liquefied gelatine. This microbe is associated with suppuration. It is found in pus, necrotic tissues, etc.

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[1] Dr. Musculus in *Comptes Rendus*, vol. lxviii.; and Dr. Sheridan Lea in *Journal of Physiology*, 1883 and 1885.

[2] See Dr. Miquel's paper in the *Annuaire de l'Observatoire de Montsouris*, 1889.

From what has been already stated in this chapter it will be seen that many micrococci are associated with wounds, abscesses, etc. Concerning the action of these microbes, Dr. W. Watson Cheyne[1] says:—

(1.) There are various kinds of micrococci found in wounds treated aseptically, differing markedly from each other in their effects on animals. They agree in growing best at the temperature of the body, and in causing acidity and sweaty smell in the fluids in which they grow. The experiments (Cheyne's) show that cultivations may be carried on in fluid media with accuracy.

(2.) The micrococci examined grew best in media exposed to oxygen gas; and they grew only with difficulty in the absence of oxygen. Dr. A. Ogston[2] stated that these micrococci were anaërobic; but there is no doubt that this statement is erroneous.

(3.) Their effect on animals was not altered by growth with or without oxygen.

(4.) The effects of these micrococci on rabbits and man were not similar, some of the most virulent forms for rabbits causing no deleterious effect in wounds in man.

(5.) The kidney is apparently an important excreting organ for microbes (Fig. 31); and microbes incapable of growing in the blood, may cause serious effects by growing in the excretory canals. This may explain some cause of pyelitis.

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[1] *British Medical Journal*, 1884.

[2] *Ibid.*, 1881.

(6.) Micrococci are always present in acute abscesses, and are probably the cause of them. In some cases, the micrococci are the primary cause of the inflammation and suppuration, as in pyæmic

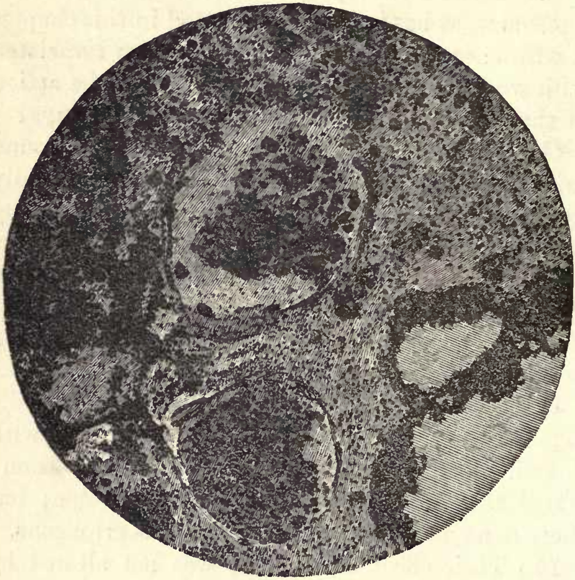

FIG. 31. SECTION OF KIDNEY CONTAINING MICROCOCCI (*after Watson Cheyne*). To the left is a mass of micrococci; to the right an inflammatory ring, and intermediately the necrotic area, infiltrated with micrococci. What are evidently remains of two kidney-tubules are seen full of micrococci and leucocytes.

× 375.

abscesses; generally, however, they begin to act after inflammation has been previously induced. This inflammation may be caused by an injury, by the absorption of chemically irritating substances from wounds, by colds, etc.

(7.) There are several different kinds of micrococci associated with suppuration.

(8.) Micrococci cause suppuration by the production of a chemically irritating substance (probably a ptomaïne), which, if applied to the tissues in a concentrated form, causes necrosis of the tissue, but, if more dilute, causes inflammation and suppuration.

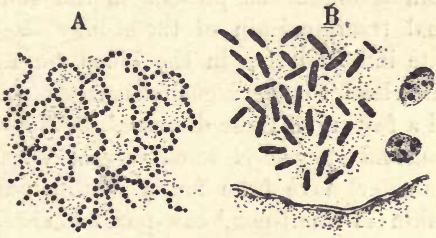

FIG. 32. MICROBES IN PURPURA.
(*Watson Cheyne.*)
A, Micrococci. B, Bacilli.
× 2500.

Micrococcus in purpura hæmorrhagica.—Watson Cheyne[1] has observed cocci (measuring 1·15 μ in diameter) in certain cases of purpura hæmorrhagica. This microbe forms colonies in the blood; and the hæmorrhages are due to the plugging of the small vessels by masses of these microbes. The microbes occur in chains (Fig. 32), and stain well with methylene blue. In another case of the same disease, Watson Cheyne found that certain bacilli plugged the vessels and gave rise to hæmorrhages. Concerning this disease, he remarks that 'we may have to do with an infective disease of which the essence is the entrance of certain specific organisms into the blood, and their growth in it. It may, however, be that in these two cases, and in others, the primary affection is something quite distinct from microbes, resulting, however, in such an altered constitution of the fluids of the body, that of the innumerable organisms present in the mouth and intestinal tract, certain of them may be able to penetrate into and live in the blood, form emboli, and thus lead to the hæmorrhages which are so marked a feature of these diseases.'

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[1] *Transactions of the Pathological Society of London*, 1884.

Micrococcus variolæ et vacciniæ.—Micrococci (0·5 μ in diameter) have been found in the lymphatics of the skin (in small-pox,[1] cow-pox, and sheep-pox [2]) in the vicinity of the pocks. The microbes were found by Cohn [3] in the lymph of vaccina and variola. No doubt they are the active agent in small-pox and cow-pox, for if the lymph is filtered through a Chamberland filter, the filtrate loses its infectious properties.

The author [4] has shown that a solution of salicylic acid acts upon vaccine lymph, and deprives it of the power of inoculation.

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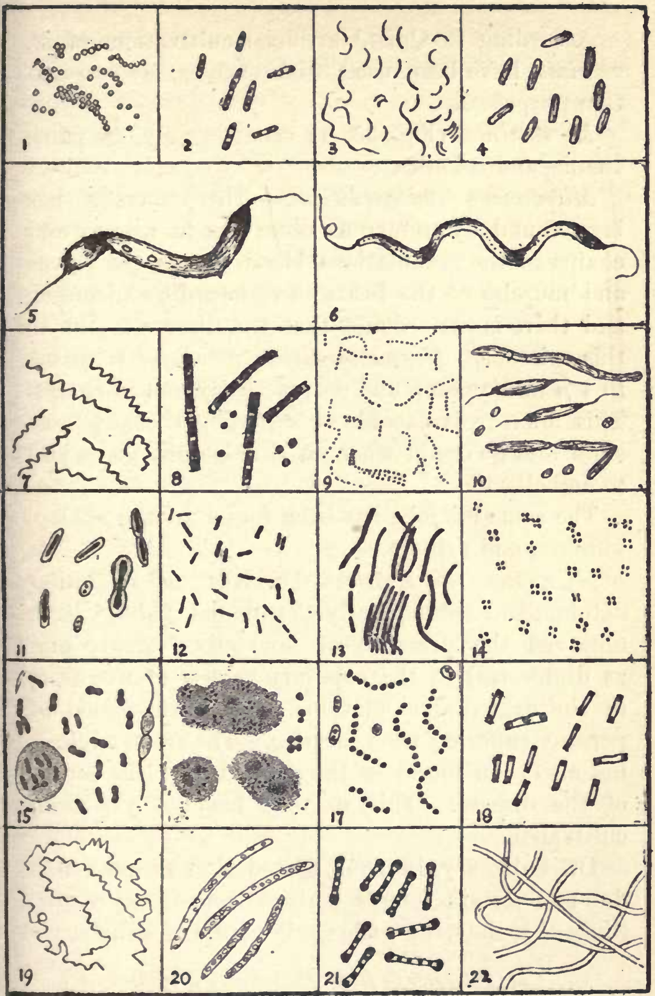
[1] Weigert in *Med. Centralblatt*, 1871.

[2] Klein in *Philosophical Transactions of Royal Society*, 1874.

[3] *Virchow's Archiv*, vol. lv.

[4] Griffiths in *Proc. Roy. Soc. Edinburgh*, vol. xiv. p. 97.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A.B. Griffiths del.

FIG. 33. VARIOUS MICROBES.

According to Quist,[1] artificial cultivations of *M. vacciniæ* have been used, with success, for vaccination purposes.

M. vacciniæ (Fig. 33, 1) occurs singly, in pairs, chains, and colonies.

Micrococcus endocarditicus.—This microbe has been found by numerous observers in masses and chains in the granulations, blood-vessels, the valves and muscles of the heart in endocarditis ulcerosa; and there is little doubt that the disease is due to this microbe. *M. endocarditicus* measures from 0·5 to 1 μ in diameter, and occurs singly and in chains. This microbe is capable of assuming the zooglœan state, and no doubt when in this state it gives rise to embolism.

The same microbe has been found in the spleen, kidneys, and urine.

Micrococcus in Measles.—Dr. Keating[2] of Philadelphia, and subsequently Cornil and Babès,[3] have observed the presence of micrococci (singly and as diplococci) in the capillary vessels of the skin, in the catarrhal exudations, and in the blood of persons suffering from measles. The same microbe has also been found in the urine during the course of the disease. This microbe has not yet been cultivated.

Dr. Salisbury, in 1862, stated that measles was due to a certain fungus derived from musty straw. Since that date, the pathogenic nature of Salisbury's straw-fungus has been generally discredited, until the year 1889, when Mr. C. Candler[1] argued in favour of Salisbury's theory—that fungus-dust from mouldy straw produces a disease resembling measles; and that this fungus-dust when introduced into the human body, develops into microbes (!). In the great epidemic of measles in Victoria during the years 1874-75, Candler states that he could not discover any instance of measles in a dwelling from which damp straw (in the form of bedding) had been excluded, but in every house where measles occurred, the presence of damp straw in the bedrooms was easily made out. There is nothing impossible in the supposition that damp straw favours the growth of *microbes*; and it might conceivably be proved by sufficient evidence that this is a favouring or even a necessary condition for the growth of the specific virus of measles. But the evidence which Candler adduces is quite inadequate to prove that the cause of measles is a fungus, since it might just as well be Keating's micrococcus or any other microbe.

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[1] *St. Petersburgh Med. Wochenschrift*, 1883.

[2] *Philadelphia Medical Times*, 1882.

[3] *Les Bactéries*, 1885.

Micrococcus gonorrhœæ.—Drs. Neisser,[2] Bokai, and Finkelstein[3] have described micrococci in the urethral discharge and the pus of gonorrhœa. These microbes (Fig. 33, 16) measure 0·83 μ in diameter, and occur singly, as diplococci, sarcinæ, and in zooglœan groups. They frequently adhere to the epithelial cells and pus-corpuscles. Dr.

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[1] *The Prevention of Measles*, 1889.

[2] *Centralblatt für d. Med. Wissensch.*, 1879.

[3] *Prager Med. Chir. Presse*, 1880.

Bockhart[1] has artificially cultivated these microbes; and has reproduced the disease by inoculation, thus proving their pathogenic character. A similar micrococcus is often found in the purulent ophthalmia of new-born infants; and it is possible that such ophthalmia is, in the majority of cases, of gonorrhœal origin.

'Aufrecht[2] reports the case of an infant twelve days old who died with suppuration of the umbilical vein and liver. The liver cells and the interlobular tissue were crowded with micrococci. These micrococci corresponded in size to *Micrococcus gonorrhœæ*, and he thinks it probable that they were derived from the vagina of the mother; during birth they might have got into the umbilical vein, there caused inflammation, and thence passed into the liver' (Klein).

Micrococus tetragonus.—This microbe is found in the sputum of patients suffering from phthisis. It is only saprophytic in man, but pathogenic in animals. Mice inoculated with a pure cultivation of this microbe die in a few days, the microbe afterwards being found in the various organs of the body. *Micrococcus tetragonus* (Fig. 33, 14) measures 1 μ in diameter, and occurs as tetrads surrounded by a hyaline membrane. This microbe forms small white points on nutrient gelatine in about twenty-four hours, which ultimately run together.

Micrococcus intracellularis meningitidis.—This microbe has been observed in the pus found at the base of brain after death in cases of acute cerebral meningitis. It occurs singly, as diplococci, chains, and zoogl≈ìa; and it grows on a mixture of agar-agar and gelatine at the temperature of the body. This microbe grows better at the surface than in the deeper layers of the medium, and gives rise to finely granular and yellowish-brown colonies. The microbe, when cultivated artificially, only remains virulent for six days; and it is said that it 'affects mice, guinea-pigs, rabbits, and dogs.' Like *M. gonorrh≈ì√¶*, this microbe 'is almost invariably found within the cells contained in the exudation.'

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[1] *Sitzungsberichte der Phys. Med. Gesell. Würzburg*, 1882.

[2] *Centralblatt für d. Med. Wissensch.*, 1883.

Micrococcus bombycis.—The cells are oval, and measure 0·5 μ in diameter. They occur singly, as diplococci and chains, and produce the 'flacherie' or 'schlafsucht'—one of the silkworm diseases. Another disease of the same larva is known as 'pébrine' 'maladie des corpuscules,' and is caused by a microbe called *Micrococcus ovatus*, which measures about 1·5 μ in diameter. *M. ovatus* is present in large numbers in the blood and organs of affected silkworms.

Micrococcus of cattle-plague.—Micrococci have been found in the blood and lymphatic glands of cattle dead of this disease. They occur singly, as chains and zoogl≈ìa, and grow rapidly in bouillon and other media at 37° C. Semmer and Archangelski[1] have shown that calves inoculated from a pure cultivation of this microbe died in seven days with all the typical symptoms of cattle-plague or rinderpest.

By successive cultivations, or by exposing cultivations for an hour to a temperature of 46° C., the virulence of this microbe is greatly reduced; and in this attenuated or weakened form it has been used for the protective inoculation of sheep and cattle.

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[1] *Centralblatt für d. Med. Wissensch.*, 1883.

Micrococcus of foot-and-mouth disease.—According to Dr. Klein, the microbe of this disease occurs singly, as diplococci, and in curved chains. 'It grows well in milk, in alkaline peptone broth, in nutrient gelatine, and in agar-agar mixture. Growing on solid material, its growth, besides being extremely slow, is very characteristic; it forms a film composed of minute granules or droplets, closely placed side by side, but not confluent. It does not liquefy nutrient gelatine, and in liquids does not form a pellicle, but nevertheless when grown on solids, its growth remains limited to the surface. It does not curdle milk, although it turns the reaction of this latter slightly but distinctly acid.' The microbe has been observed in the vesicles of sheep suffering from the disease.

Micrococcus septicus.—The cells are 0·5 μ in diameter, and occur singly, as diplococci and chains. Colonies are produced very slowly on nutrient gelatine; they are seen as minute dots on the fourth and fifth days in plate and tube cultivations. They are fatal to mice, rabbits, etc.; the vessels in the various organs become plugged with these microbes, this ultimately forming purulent or necrotic foci. *M. septicus* is present in soil.

Micrococcus in gangrene.—Small oval micrococci have been found in gangrene of the lungs. They live in colonies, form zooglœa, and grow on nutrient gelatine, giving rise to the characteristic but offensive gangrenous odour. The same microbes have been observed in various gangrenous tissues, and also in the blood of patients suffering from 'Clou de Biskra' or 'Bouton d'Alep,' which excite gangrene when injected into rabbits.

Micrococcus perniciosus.—According to Wolff[1] this microbe is the cause of a disease of the grey parrot. The cells measure 0·8 μ in diameter, and occur singly, in chains and zoogl[oe]a. They produce nodules in the liver, lungs, spleen, and kidneys; but inflammation around the nodules is entirely absent. The microbes also occur in the blood. The disease is said to be fatal to 80 per cent. of the grey parrots imported into Europe.

Micrococcus insectorum.—This microbe occurs in the digestive organs of the chinck-bug (*Blissus leucopterus*); and is probably the cause of an infectious disease of this insect. The cells are obtusely oval (0·7 to 1 μ long × 0·55 μ broad), and occur singly, in pairs, chains, or zoogl[oe]a. They may be cultivated in bouillon.

Micrococcus of Tissue Necrosis in Mice.—Dr. R. Koch observed that a certain micrococcus, isolated from putrid fluids, when injected into the ear of a mouse, gave rise to tissue necrosis and death in about three days. The microbe was not found in the blood and internal organs. The cells measure 0·5 μ in diameter, and occur in chains and zoogl[oe]a.

Micrococcus in whooping-cough.—Whooping-cough is undoubtedly an infectious disease, and, according to Dr. Bürger,[1] oval micrococci are often present in the pearly phlegm ejected by patients suffering from this disease. They have not yet been cultivated.

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[1] *Virchow's Archiv*, 1883.

Micrococcus in pernicious anæmia.—According to Frankenhäuser[2] the blood of pregnant women suffering from pernicious anæmia contains a large number of micrococci which appear to be of a pathogenic character. These micrococci are comparatively of large size, 'about one-tenth of the broad diameter of a red blood-corpuscle.' These microbes have not been cultivated.

Micrococcus of Nitric Fermentation.—Mr. R. Warington, F.R.S.,[3] has recently isolated from soil a micrococcus which converts nitrites into nitrates. But this micrococcus, as well as Frankland's nitrous bacillus, will be described later, *i.e.* under the heading of 'the microbes of the soil.'

Micrococci in Pyæmia and Septicæmia.—A considerable number of micrococci (from 0·1 to 1·0 μ in diameter) have been found in various organs, blood, etc., in pyæmia and septicæmia in the lower animals and in man.[4]

Micrococci have also been described in hæmophilia neonatorium, in ozæna, in acute yellow atrophy of the liver, in closed abscesses, and in many other diseased conditions.

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[1] *Berlin Klin. Woch.*, 1883. See also the Appendix.

[2] *Centralblatt für d. Med. Wissensch*, 1883.

[3] *Journal of Chemical Society*, 1891, pp. 484-529.

[4] See Dr. Watson Cheyne's papers in the *British Medical Journal*, Sept. 20, 27, Oct. 4, 1884, and July 31, 1886; also Dr. Crookshank's *Manual of Bacteriology* (2d ed.)

BACTERIA.

Bacterium termo.—The cells are oblong and measure 1·5 μ in length and about 0·5 μ in breadth (Fig. 33, 15). Each cell is surrounded with a thick membrane of cellulose ($C_6 H_{10} O_5$) and a flagellum at each end. Dr. W. H. Dallinger, F.R.S., has measured the diameter of the flagellum of this microbe, and he finds that it is the $\frac{1}{204700}$th of an inch, or expressed in decimals 0·00000488526 inch.[1] *B. termo* is one of the commonest forms in water and putrefying fluids, but it always disappears when putrefaction terminates, in fact it has been called the microbe of putrefaction. It has remarkable powers of vitality; it is most active between 32° and 36° C.; at a temperature below 5° C. and above 46° C. it does not produce putrefaction in putrescible fluids; however, above 50° C. it is killed, but even at so low a temperature as $-110°$ C. this microbe is not destroyed.[2] It grows well on bouillon, agar-agar, etc., and after several days' incubation a pellicle is formed on the fluid medium. When grown on solid agar-agar it imperfectly liquefies the medium, and gaseous products are generated. On sterilised potatoes *B. termo* produces a slimy grey colony. It occurs singly, in pairs, and zooglœa, and it is readily obtained by placing a piece of meat in water kept in a warm place for a few hours. It may be remarked that this microbe has been considered to be only a phase-form of a protean species.

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[1] *Journal of Royal Microscopical Society*, 1878, p. 174.

[2] Giglioli's *Fermentive Microbi*, p. 55.

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Bacterium lineola.—This microbe resembles *B. termo* in form and movement, only it is much longer and thicker than that microbe. Each cell measures from 3 to 5 μ in length and 1·5 μ in breadth, and is provided with two flagella, one at each end of the cell. This microbe occurs singly, in pairs, in zoogl\u0153a, but never in chains or rosaries. It is found in well-water and stagnant water, where no distinct putrefaction is going on; it forms pellicles on sterilised potatoes and various infusions.

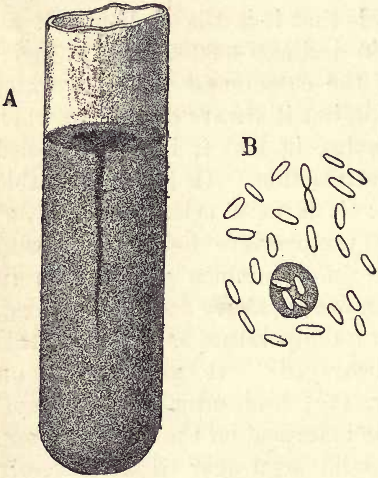

FIG. 34. BACTERIUM ALLII.
A, Growing on agar-agar.
B, The microbe isolated.

Bacterium allii.—During the year 1887 the author[1] discovered this microbe in the greenish slime of diseased or putrefying onions and allied plants. It measures from 5 to 7 μ in length and 2 μ in breadth, and occurs singly, in pairs, and forms zoogl\u0153a. It has been named *Bacterium allii* because it was originally found on *Allium cepa* (the onion). *Bacterium allii* grows tolerably well on nutrient agar-agar, and produces a bright green pellicle on the surface of the nourishing medium (Fig. 34). The green pigment is soluble in alcohol, and an alcoholic solution gives an absorption spectrum, consisting of a band extending from the extreme violet to the blue part (nearly to the Fraunhofer line F) of the spectrum. There is also an absorption band in the green, and one in the yellow, part of the spectrum. The end of the band in the yellow is exactly in the same position as the D line in the solar spectrum. *Bacterium allii* forms an alkaloid or ptomaïne from albuminoid molecules. This ptomaïne has the same chemical composition as hydrocoridine ($C_{10} H_{17} N$).[1] Besides the pigment and ptomaïne, small quantities of sulphuretted hydrogen gas are liberated from the medium on which the microbe lives. The sulphuretted hydrogen was proved by the black stain (PbS) produced upon paper impregnated with a solution of lead acetate, and also by the yellow stain (CdS) produced on cadmium paper ($CdCl_2$). *B. allii* is best stained with gentian violet. The vitality of this microbe is remarkable,[2] for it still retains its vitality when exposed, in a dry state, to a temperature of 32° C. for six months. A pure culture of this microbe exposed to $-15°$ C. for three days proved that it was not killed; but it was killed after fourteen days' exposure at the same temperature. An E.M.F. of 3·3 volts killed *B. allii* in ten minutes.

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[1] *Proceedings of Royal Society of Edinburgh*, vol. xv. p. 40.

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[1] See Dr. Griffiths' papers in *Comptes Rendus de l'Académie des Sciences,* tome 110, p. 416; and *Centralblatt für Bakteriologie und Parasitenkunde,* Bd. vii. (1890), p. 808.

[2] *Proceedings of Royal Society of Edinburgh,* vol. xvii. pp. 262-264.

The microbe in question is quite distinct from the *bacillus* (giving a green fluorescence) which Heraëus obtained from soil.[1] The bacillus of Heraëus converts urea into ammonia, while *Bacterium allii* has no such action, for it decomposes albuminoids (vegetal and animal) with the formation of a ptomaïne among other products.

Bacterium aceti.—This is the microbe which causes the acetic fermentation according to the well-known reaction:—

$$C_2H_5OH + O_2 = H_2O + CH_3COOH.$$

It is about 1·5 μ in length, and occurs singly, in long chains, and forms a pellicle on the surface of the nutritive fluid. Although Pasteur maintained that *B. aceti* was the cause of the acetic fermentation, and Cohn[2] observed the microbe largely in sour beers, yet not until the commencement of 1886 could any one say with certainty that this microbe was the real cause of the acetic fermentation. In that year Mr. A. J. Brown[3] prepared *pure* cultivations of the microbe in question, and found that it does convert alcohol into acetic acid or vinegar. The author[4] entirely indorses the correctness of Brown's researches. After obtaining pure cultivations of *B. aceti* by the fractional and dilution methods, it was found that these cultivations, when used to inoculate sterilised ethyl alcohol (6 per cent.) gave acetic acid in abundance.

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[1] *Zeitschrift für Hygiene*, 1886.

[2] *Biol. d. Pflanzen*, Bd. ii. p. 173.

[3] *Journal of Chemical Society*, 1886, p. 172.

[4] *Proceedings of Royal Society of Edinburgh*, vol. xv. p. 46.

Bacterium lactis.—The cells measure from 1·5 to 3 μ in length, and are constricted in the centre like the figure 8. They occur singly, in long chains, zooglœa, and like *B. aceti*, they are motile. *Bacterium lactis* is the cause of the lactic fermentation or the souring of milk. The sugar of milk or lactose is converted into lactic acid by the growth of this microbe (Lister[1]). Unlike *B. aceti*, this microbe is anaërobic. *B. lactis*, along with other microbes, plays an important part in the preparation of sauerkraut; and Dr. Baginski has recently shown that it produces a powerful reducing action in pure cultivations, where the nutrient fluid was coloured with methylene blue.

Bacterium decalvans.—This microbe was discovered by Dr. G. Thin[2] in the roots of the hair in cases of *Alopecia areata*; and he supposes that it penetrates downwards between the root-sheath and the hair, then passes through the cuticle of the hair, and ultimately ascends within its substance, causing it soon to fall off. It measures 1·6 μ in length, and occurs usually in pairs.

Bacterium choleræ gallinarum.—This is the microbe of fowl cholera, and it is found in large numbers in the blood and organs of fowls dead of this disease. It measures 1·2 to 1·5 μ in length, and the ends are always stained more deeply than the middle part. *B. choleræ gallinarum* is easily cultivated in chicken broth (neutral) at 25°—35° C., and when fowls are inoculated with a drop of this culture they always die with the characteristic symptoms of the disease. If a culture of the microbe is kept for two or three months its virulence is lessened; and an attenuated virus has been successfully used by Pasteur in the protective inoculation of fowls against this disease. This microbe is pathogenic in rabbits as well as fowls, but guinea-pigs have an immunity; it is aërobic, and is cultivated in contact with sterilised air or in aërated fluids. In fact, 'its toxic effect has been supposed to be due to the abstraction of oxygen from the blood, producing asphyxia.' *B. choleræ gallinarum* grows on gelatine as small, round, white colonies with lemon-yellow centre. It grows on potatoes at 37° C., producing yellow-grey drops. M. Duclaux[1] has shown that this microbe produces a ptomaïne; but when the ptomaïne is separated, by filtration through porous porcelain from its microbe it does not produce fowl cholera; for it causes a passing sleep, which does not generally end fatally. From this fact the conclusion may be drawn that in fowl cholera the microbe is essentially the active agent in producing the disease.

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[1] *Transactions of the Pathological Society*, 1878.

Proceedings of Royal Society, vol. xxxiii. p. 247.

Bacterium pseudo-pneumonicum.—This microbe forms greyish-white layers in test-tube cultivations; while on gelatine plates the colonies appear as white dots. It grows on sterilised potatoes at 37° C., giving rise to a white, viscid layer; it measures 1·16 μ in length, and 0·8 μ in breadth, and requires air for its growth. It is only slightly pathogenic. *B. pseudo-pneumonicum* occurs in pus taken from abscesses.

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[1] *Ferments et Maladies.*

Bacterium xanthinum.—This bacterium is the cause of 'yellow milk,' which at first is acid, but soon becomes alkaline. The pigment produced by this bacterium is soluble in water, and insoluble in alcohol and ether. *B. xanthinum* measures from 0·7 to 1·0 μ in length, and forms colonies on potatoes.

Bacterium septicus agrigenum.—This microbe occurs in soils, and measures from 2 to 3 μ in length. On plate cultivations it produces colonies of a greyish-yellow colour, with a yellowish-brown centre. This microbe is fatal to mice, rabbits, and guinea-pigs. It multiplies rapidly in the blood, and it adheres to the red blood corpuscles.

Bacterium coli commune.—This bacterium measures 1·7 μ in length and 0·4 μ in breadth, and it occurs in the fæces of infants fed on human milk. It grows on nutrient gelatine, forming granular colonies of a yellowish colour. It is fatal to rabbits and guinea-pigs, causing violent diarrhœa and fever.

Bacterium fœtidum.—This microbe was discovered by Dr. Thin[1] in the alkaline serous exudation from the soles of the feet of a person who suffered from profuse sweating of the feet. It produces a fœtid odour. which is also observable in artificial cultures of this microbe. *B. fœtidum* occurs singly, in pairs, and leptothrix threads. This microbe appears to be identical with Rosenbach's *Bacillus saprogenes*; and it is readily cultivated in agar-agar and blood-serum.

Bacterium Neapolitanum.—This microbe occurs as short-rods with rounded ends, measuring about 1 μ in length. On nutrient gelatine it forms circular colonies, which, however, become irregular, granular, refractive, and of a yellow colour. It was isolated from certain cases of cholera at Naples; but it has nothing to do with the disease, for it is only saprophytic in man.

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[1] *Proceedings of Royal Society*, vol. xxx. p. 473.

Bacterium septicum sputigenum.—This microbe is identical with Sternberg's *Micrococcus Pasteurianus* and Fränkel's *Bacillus septicus sputigenus.* It is present in human saliva, and occurs as short rods, frequently joined together in chains of five, six, or seven links. It is usually obtained from the rusty sputum of pneumonic patients and from severe cases of empyæmia. It grows well in bouillon and on agar-agar at 34° C., but slowly on gelatine plates. The colonies are granular and white. This microbe is pathogenic in rabbits, mice, and guinea-pigs, but fowls and dogs have an immunity. Dr. Watson Cheyne[1] says that it 'apparently loses its virulence when cultivated outside the body. The blood of rabbits which have died of this microbe is very virulent, a small quantity being sufficient to set up the disease in a fresh animal, but when grown in meat-infusion, agar-agar, etc., it rapidly (in three or four days, unless re-inoculated into fresh material) loses its virulence, and the dose necessary to cause death increases as the cultivation becomes older. When it does not cause death it may produce a slight local effect, and such animals are apparently protected from a subsequent inoculation with virulent material. The animals often do not die for three or four days after the injection, and generally exhibit nervous symptoms, sometimes ending in paraplegia.'

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[1] *British Medical Journal*, July 31, 1886.

Bacterium indicum.—Is an aërial microbe rod-shaped with rounded ends. On nutrient agar-agar it produces a scarlet-coloured growth, but after some days the growth loses its bright colour, and becomes purplish, like an old cultivation of *Micrococcus prodigiosus.* On gelatine this microbe liquefies the medium, and colours it scarlet. It also grows well on the surface of potatoes.

Bacterium merismopedioides.—Each rod measures from 1 to 1·5 μ in thickness. It was first observed by Dr. Zopf in the river Panke, Berlin, and is said to divide into long and short rods, and finally into cocci. This microbe also exists in zooglean form.[1]

Bacterium Zopfii.—This bacterium, which was discovered by Kurth, measures from 2 to 5 μ in length, and from 0·7 to 1 μ in breadth. It is motile, and occurs in long threads. It grows on gelatine-plates, developing into thread-like growths in about thirty-six hours. This microbe was first isolated from the intestine of fowls.

Bacterium oxytocum perniciosum.—First isolated from sour milk. Each rod has rounded ends, and forms yellowish colonies on gelatine plates. Needle cultures have the characteristic nail appearance. In milk this microbe produces curdling and an acid reaction. It measures 1 μ in length; and in large doses it is pathogenic in rabbits.

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[1] Zopf, *Die Spaltpilze* (1885); and *Die Pilze* (1890).

Bacterium phosphorescens.—The cells of this microbe are almost circular, being from 1·3 to 1·9 μ long, and 1·1 to 1·7 μ broad; each cell is motile, and surrounded by a gelatinous membrane. It is readily cultivated on fish broth containing a small quantity of peptone; it grows slowly at the ordinary temperature in peptonised gelatine, or in peptonised gelatine containing 2 per cent. of glucose, but only at the surface, and the property of emitting light depends on the presence of oxygen.[1] It also grows well in 2, 3, and 4 per cent. solutions of sea-salt, containing 0·25 per cent. of peptones. On shaking, the phosphorescence becomes intensified, but on cooling to 0° C. its intensity is somewhat diminished. The phosphorescence disappears when the solution is heated to 35° C. for a few minutes, but re-appears on cooling; it is, however, completely destroyed by heating at 35° C. for fifteen minutes.

After two or three weeks the culture solutions become yellowish, and gradually lose their phosphorescence; after several weeks phosphorescence ceases entirely, but the microbes do not die. The phosphorescence is most probably caused by ferment action in the presence of oxygen. This microbe forms colonies on the surface of agar-agar, gelatine, and potatoes, and also grows in urine and milk.

Bacterium Pflügeri.—This microbe is the most phosphorescent of all the light-emitting bacteria. It is distinguished from the preceding form by not emitting light with peptone and maltose, but it emits light with peptone and glucose. It measures from 1·5 to 1·9 μ in length, and from 1·3 to 1·7 μ in breadth; these rods have rounded ends, and appear to divide exceedingly rapidly. The bacterium is motionless, and occurs singly, and sometimes in short chains. 'On plates prepared with peptone gelatine, to which a small quantity of glucose, and from 2 to 3 per cent. of common salt have been added, the microbe develops luxuriantly, giving rise to small, white, mother-of-pearl-like colonies, about the size of a pin's head, with no surrounding zone of liquefied gelatine.' This microbe is readily obtained by placing fresh cod or herring (with *moist* surfaces) between a couple of plates, and kept at about 20° C. for twenty-four or thirty-six hours. At the end of this time small phosphorescent points or dots are seen to glow on the surfaces of the fishes. These dots are colonies of the microbe in question.

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[1] See Hjelt's *General Organic Chemistry* (English translation), p. 94.

Bacterium Fischeri.—Unlike the preceding phosphorescent microbes, *B. Fischeri* liquefies peptonised gelatine; and by the addition of a *small* quantity of sugar the intensity of the phosphorescence is increased. The microbe is motile, and occurs singly and in short chains. It grows on agar-agar at a low temperature (from 0° C. to 15° C.).

Bacterium Balticum.—Like the preceding microbe, *B. Balticum* was found in the waters of the Baltic, and also liquefies peptonised gelatine.

The four forms of phosphorescent bacteria cannot develop their light-emitting functions to their highest point without the presence of some substance from which carbon may be easily obtained, such as glycerol, glucose, asparagine, sugar, etc., as well as peptone. For this reason they have been termed peptone-carbon-bacteria. Beyerinck,[1] who has recently studied these microbes, states that they are best cultivated in fish broth with sea-water, to which are added 1 per cent. of glycerol, 8 per cent. of gelatine, and $\frac{1}{4}$ per cent. of asparagine.

Photo-Bacterium Indicum.—This microbe occurs in the West Indian Sea. It liquefies gelatine very rapidly; and the greatest intensity of light is given off when the culture is kept between the temperatures of 30° and 35° C.

Bacterium luminosum.—This microbe, which is most active at about 15° C., is found in the North Sea. Both the preceding and the present microbes give off light in peptonised gelatine without requiring the presence of sugar or any other carbohydrate, consequently they have been termed peptone-bacteria.

In all these bacteria (phosphorescent) the development of luminosity is constantly accompanied by the transition of peptones into organised, living matter, under the influence of free oxygen, with or without the concurrence of another compound containing carbon.

Certain other bacteria, although they do not emit light, are influenced by it, among these are the two following microbes:—

Bacterium chlorinum.—The cells are from 2 to 3 μ in length, and are motile. This microbe accumulates in the light, but only when oxygen is absent.

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[1] *Transactions of Royal Academy of Sciences of Amsterdam,* 1890.

Bacterium photometricum. — According to Dr. T. W. Engelmann,[1] this microbe is influenced by light; in fact, its movements are stated to depend on light. It produces a red pigment, but the amount of pigment formed varies with the action of light. Different coloured lights affect this bacterium differently, the most powerful being the ultra-red, the yellow, and part of the green.

Bacterium crassum sputigenum.—This microbe was originally isolated from sputum; it also occurs in the 'fur' scraped from the tongue. It measures 1 μ in length and 0·8 μ in breadth. Colonies on gelatine plates appear as grey, viscid drops, and in needle cultures develop a nail-shaped growth. This microbe is fatal to mice, rabbits, and dogs.

Bacterium pneumonicum agile.—This is the microbe of vagus pneumonia of rabbits. The cells are short thick rods, which occur singly or in chains of three or four. This bacterium forms dark granular colonies on gelatine, which subsequently liquefies. It also grows on blood serum, bouillon, and potatoes. The growth on potatoes spreads very rapidly over the whole surface as a red layer. Pure cultures of this microbe are fatal to rabbits.

Bacterium violaceum.—This microbe was discovered by Bergonzini,[2] and it measures from 2 to 3 μ in length, and from 0·6 to 1 μ in breadth. It occurs on egg-albumin, forming a violet pigment. This pigment is insoluble in water, and soluble in alcohol and ether. It is said that ether dissolves out a red-violet pigment, and alcohol a deep blue one.

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[1] *Pflüger's Archiv*, vol. xxx. p. 95; *Revue Internat. Science*, tome ix. (1882), p. 469.

[2] *Ann. Soc. Nat. Moden.*, vol. xiv.

Bacterium brunneum.—'Motile rods, producing a brown colour. They were observed on a rotting infusion of maize.'

Proteus vulgaris.—This bacterium is found in abscesses, putrefying organic matter, meconium-fæces, and in water. The rods are from 1·25 to 3·75 μ in length, and about 0·6 μ in breadth. The threads or chains are usually twisted and convoluted, and, according to Hauser,[1] involution forms are found—spherical bodies about 1·6 μ in diameter. This microbe grows rapidly in nutrient gelatine, causing liquefaction of the gelatine. In test-tube cultivations, the fluid gelatine, which is at first turbid, becomes subsequently more or less clear in the middle, with a deposit of flocculi at the bottom, and a slight turbidity at the top. Growing on gelatine plates, this microbe rapidly forms greyish masses, which consist of motile and swarming colonies. After forty-eight hours' growth a foul odour and an alkaline reaction are developed. This microbe is pathogenic, and produces abscesses or death according to the dose or quantity of the microbian culture injected into the animal; with rabbits and mice inoculation does not cause any effect, but the injection of quantities varying from $\frac{1}{10}$ to $\frac{3}{10}$ cc. causes death. On this point Watson Cheyne[2] states 'that $\frac{1}{10}$ cc. injected into the muscular tissue was a fatal dose, indeed $\frac{1}{20}$ cc. almost invariably killed, though some animals survived it: $\frac{1}{40}$ cc. always caused an extensive abscess, of which the animals usually died in six to eight weeks. Doses of less than $\frac{1}{500}$ cc. did not produce any effect. We have thus three results, according to the dose employed. A small dose (below $\frac{1}{500}$ cc.) produced no effect; from $\frac{1}{120}$ to $\frac{1}{40}$ cc. caused abscesses, while above $\frac{1}{20}$ cc. caused death in from twenty-four to thirty-six hours. Further, the size of the abscess depended apparently also on the dose, $\frac{1}{500}$ cc. causing only a slight trace of pus, which disappears, while $\frac{1}{40}$ causes a large and spreading abscess, ultimately resulting in the death of the animal; and the intermediate doses produce intermediate effects. On several occasions I have diluted the cultivation considerably, and made plate-cultivations from this diluted material, in order to ascertain the number of bacteria present by counting the number of colonies which developed. The result is that on an average 1 cc. of gelatine cultivations contained 4,500,000,000 bacteria. Thus, doses up to 9,000,000 produced no effect; from 9,000,000 up to 112,500,000 caused abscesses, and above 225,000,000 caused death. It is difficult to understand the influence of dose in producing these effects, but the following seems to be a fair supposition. Rabbits are not very susceptible to the action of this bacterium; in other words, in the struggle for existence between the bacteria and the cells which follows the introduction of this bacterium, the victory will, in most cases, remain with the cells, and the bacteria will disappear. If, however, along with the bacteria, a large quantity of their products (ptomaïnes) are introduced, these products interfere with the action of the cells, and enable the bacteria to get a foothold. If a large number of bacteria are introduced at one place they grow for a time till attacked by the cells, and each produces a small quantity of poisonous material. Where the number of bacteria is very large this material destroys the tissues in the neighbourhood, and enables the bacteria to spread over a large area before the layer of cells formed around them is able to form a barrier against their progress. The extent to which they spread—in other words, the size of the abscess which results—must, therefore, depend firstly on the number of bacteria and the quantity of products introduced in the first instance; and, secondly, on the vitality of the animal. It may be that a very large amount of organisms is introduced in the first instance, producing such an amount of poison as to kill the animal in a few hours.' The investigations of Watson Cheyne, as well as those of Hauser, undoubtedly prove that a ptomaïne of poisonous properties is formed by these bacteria (*Proteus vulgaris*), but there can be no doubt, also, that these bacteria themselves are truly pathogenic for rabbits under proper conditions. Watson Cheyne has also shown that when *Proteus vulgaris* is grown in bouillon it acts with less virulence than when it is grown in nutrient gelatine.

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[1] *Ueber Fäulniss-Bacterien*, 1885.

[2] *British Medical Journal*, July 31, 1886.

Proteus mirabilis.—This bacterium is something like the preceding microbe, although somewhat shorter. It liquefies gelatine much slower than

Proteus vulgaris, and forms granular colonies of a brownish colour. It also forms zooglœa.

Proteus Zenkeri.—This motile bacterium is 1·65 μ in length and 0·4 μ in breadth. In plate-cultivations it gives rise to greyish colonies, but no zooglœa are formed. There is only a very slight liquefaction of the gelatine, and no odour is given off from cultures on gelatine or blood serum; but there is a strong smell given off when the microbe is cultivated in bouillon.

BACILLI.

Bacillus beribericus.—This microbe was discovered by De Lacerda[1] in the blood of patients suffering from the disease known as beri-beri, kakke, etc. It occurs singly in long chains and produces spores. When cultivated in bouillon and then injected into rabbits this microbe is said to produce all the symptoms of beri-beri. The disease is characterised by anæmia, anasarca, degeneration of the muscular tissues, numbness, pain and paralysis of the extremities; and one of its chief habitats is in Japan. It is prevalent in the Malay Archipelago, the Molucca Islands, New Guinea, Burmah, Siam, Ceylon, and India (south and east); and it is endemic as well as epidemic in other parts of the world.

According to Prof. B. H. Chamberlain,[2] 'kakke [*i.e.* beri-beri] is the national scourge of Japan, and attacks with special frequency and virulence young and otherwise healthy men—women much less often.' De Lacerda believes that the bacillus is derived from rice which has undergone a peculiar alteration.

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[1] *Lancet*, February 9, 1884, p. 268.

[2] *Things Japanese*, 1890, p. 188 (Kegan Paul, Trench, & Co.).

The epidemic spread of this disease is probably influenced by climate, and seems to coincide with conditions of high atmospheric moisture and extreme thermometric variations.[1]

Bacillus alvei.—This microbe produces the disease known as 'foul-brood' of bees, and it has been thoroughly investigated by Cheshire and Cheyne.[2] It measures 4 μ in length and 0·5 μ in breadth, and the oval spores which it produces measure 2·1 μ in length and 1·7 μ in breadth. *B. alvei* is present in the blood and juices of the larvæ, drones, workers, and queens, and is

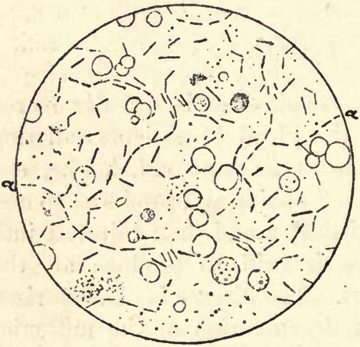

FIG. 35. BACILLUS ALVEI.
(*After Cheshire and Cheyne.*)
A, leptothrix forms (× 600).

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[1] For further information see Dr. Felkin's paper in *Proceedings of Royal Society of Edinburgh*, vol. xvi. p. 291; Dr. E. Baelz's paper in *Mittheil. Deuts. Gesellschaft für Natur- und Völkerkunde Ostasiens*, Bd. iii. p. 301; Dr. Anderson's paper in *Transactions of Asiatic Society of Japan*, vol. vi.; Dr. Wernich's *Geographisch-medicinische Studien;* Dr. Scheube's *Die Japanische Kak-ke;* and the Japanese reports by Drs. Takaki and Miura.

[2] *Journal of Royal Microscopical Society*, 1885, p. 582.

also present in the ova. Numbers of this microbe are seen moving backwards and forwards in the blood, etc., of larvæ attacked with the disease. Leptothrix forms of the microbe are common when the disease is in rapid progress; these sometimes measure 250 μ in length (Fig. 35). In the juices of the larval bee during life these bacilli do not produce spores, although after death spores abound. In test-tube cultivations the bacilli grow both on the surface of the gelatine[1] and along the needle-track. At the surface the bacilli form a delicate ramifying growth, and along the track whitish irregular-shaped masses appear, which slowly increase in size and run together. In a few days processes are seen to shoot out from these masses, which may extend through the gelatine for long distances from the track, being thickened at various parts, and clubbed at the ends. If only a very few bacilli are introduced with the needle, a beautiful and characteristic growth is obtained, for by this

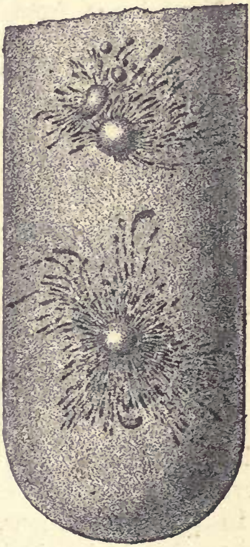

FIG. 36. BACILLUS ALVEI. (*Cheshire and Cheyne.*)

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[1] The best growth in gelatine is obtained at about 20° C.

means groups of bacilli become planted at a considerable distance from each other (Fig. 36). This appearance is quite characteristic of *B. alvei*, and is not seen in the cultivation of any other microbe. 'The bacilli of anthrax and of mouse septicæmia also spread out from the needle track, but the appearance of their cultivation is quite different. In anthrax delicate threads, not clubbed, shoot out from the track, soon anastomosing with other threads and forming a delicate network throughout the gelatine. In mouse septicæmia the appearance is that of a delicate cloudiness spreading through the gelatine. These 'foul-brood' bacilli, growing in this material, render it liquid after a time, the liquefaction beginning at the surface and only spreading slowly downwards, but ultimately the whole tube becomes liquid. The liquid becomes yellowish in colour after a time, and gives off an odour of stale, but not ammoniacal, urine. This colour and odour are distinctive of the diseased larvæ.'

In plate-cultivations, the bacilli grow out in series of rods in single file, or in rows of several side by side. The processes which are formed have a tendency to form curves and circles. Later on, the gelatine in the vicinity of the bacilli becoming liquid, forms a series of channels in which the bacilli move backwards and forwards.

They grow most rapidly on the surface of nutrient agar-agar, forming a whitish layer, but the ramifying processes seen on the surface of gelatine do not occur, or only very imperfectly, in agar-agar.

Here the bacilli arrange themselves apparently side by side, and producing spores in this position, we have as a result, after a few days' cultivation, long rows of spores lying side by side, with here and there an adult bacillus.

In milk they grow well at the body temperature, and in a few days cause coagulation of the milk; and on potatoes they form a dryish yellow layer. These bacilli also grow in blood serum and in bouillon.

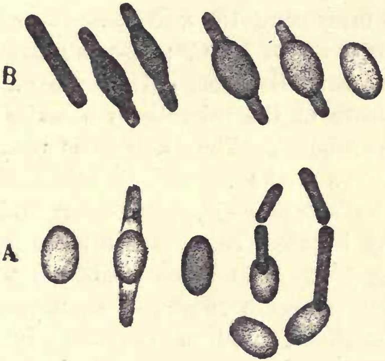

FIG. 37. BACILLUS ALVEI.
(*Cheshire and Cheyne.*)
A, Passage of spore into bacillus condition.
B, Passage of bacillus into spore condition.

B. alvei does not grow below 16° C.; but it grows most rapidly in cultivating media kept at the body temperature. Cheshire and Cheyne sprayed a cultivation of the bacillus in milk over a honeycomb containing a healthy brood of larval bees, and succeeded in reproducing the disease known as 'foul-brood.' They also succeeded in infecting adult bees by feeding them with material containing these bacilli.

This microbe is best stained with methyl violet; but the spores resemble the spores of other microbes in not taking on the stain. Fig. 37 represents the passage of a spore into the bacillus condition, and *vice versâ*.

Bacillus of Grouse Disease.—Dr. Klein[1] has recently proved the microbian nature of grouse disease. The disease, which is infectious, is caused by a bacillus measuring 1·6 × 0·6 μ. It grows well on agar-agar at 36° to 37° C.[2]; also on nutrient gelatine and in alkaline bouillon. Klein proved the pathogenic nature of the microbe by a series of inoculation experiments. The bacillus is readily stained by Weigert's method.

Bacillus subtilis.—The hay-fever microbe was originally isolated from an infusion of hay. It measures 6 × 2 μ, and has slightly rounded ends. This bacillus occurs singly, in short chains, in leptothrix filaments, and in zooglœa. It forms oval spores (1·2 × 0·6 μ); but spore-formation occurs only when there is an ample supply of air; nevertheless it is independent of any deficiency of nourishing material (Klein). The bacilli when single possess one or two flagella (Fig. 33, 10). 'The bacilli form a dense resistant pellicle on the surface of the nourishing medium, and in this copious spore-formation takes place. If shaken when growing in a fluid the pellicle falls to the bottom, and soon a new pellicle is formed.' This microbe may be readily obtained by exposing a previously sterilised infusion of hay to the atmosphere for a short time: the spores being always present in the air. On plate-cultivations, white rounded colonies formed, which frequently give rise to radiating processes. On potatoes and agar-agar *B. subtilis* forms a moist, cream-coloured layer, which ultimately becomes granular and dry. It grows on blood serum and nutrient gelatine, both of which it liquefies. *B. subtilis* is a motile microbe, and is best cultivated at a temperature of about 30° C. This microbe can withstand a temperature of $-18°$ C.;[1] and its spores have been proved to have a remarkable power of resisting the influence of high degrees of heat. For instance, a short exposure to 100° C. does not destroy the vitality of the spores. However, an E.M.F. of 2·72 volts destroys both the spores and bacilli.[2] The action of ozone[3] on both the spores and bacilli is that they are completely destroyed; this fact explains the absence of this and other microbes in the air at sea—the latter containing an appreciable amount of ozone.

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[1] *Centralblatt für Bakteriologie und Parasitenkunde*, Bd. vi. pp. 36 and 593; Bd. vii. p. 82; and Bd. ix. p. 47.

[2] That is, in from two to four days.

Bacillus ethaceticus.—This small bacillus ($1·5$ to $5·1 \times 0·8$ to $1·0\ \mu$) was discovered by Dr. P. F. Frankland, F.R.S.,[4] and has the power of decomposing solutions of mannite, glucose, sucrose, lactose, starch, glycerol, and calcium glycerate. It has no fermentive action on dulcite, the isomer of mannite, which thus furnishes a very striking instance of the selective power of microbes between the most closely allied isomeric bodies. The products of the fermentation of the above-mentioned compounds are essentially alcohol and acetic acid, with a small and variable proportion of formic acid, together with a trace of succinic acid. Frankland[1] represents the decomposition of glyceric acid (calcium glycerate) by this microbe as follows:

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[1] Griffiths in *Proceedings of Royal Society of Edinburgh*, vol. xvii. p. 263.

[2] Griffiths, *ibid.*, vol. xv. p. 45.

[3] Griffiths' *Researches on Micro-Organisms*, p. 184.

[4] *Proceedings of Royal Society of London*, vol. xlvi. p. 345.

$$5C_3H_6O_4 = C_2H_5OH + 4CH_3COOH + H_2O + 3H_2 + 5CO_2.$$

The alcohol and acetic acid are produced approximately in the proportion of one molecule of alcohol to four molecules of acetic acid.

Bacillus butyricus.—This is the microbe of the butyric fermentation; and it is found in the cells of laticiferous plants, in milk, and in decaying-plant infusions, etc. *B. butyricus* is morphologically like *B. subtilis,* but distinguished by the fact that at certain times it contains starch in its cells. It measures from 3 to 10 μ in length and 1 μ in breadth: it frequently forms chains, and gives rise to well-developed spores. When spore-formation is about to take place the protoplasm of the cell becomes granular, and at certain points gives rise to oval spores. This microbe grows on gelatine-plates, in the deeper layers of the medium, as yellow or brown colonies of a granular appearance; and ultimately the gelatine is liquefied. On agar-agar it forms a viscid yellow layer; while in test-tube cultivations it liquefies the gelatine which becomes cloudy. *B. butyricus* grows best between 35° and 40° C. It is the cause of the rancidity of butter and the ripening of cheese. It decomposes cellulose, and hence it is of great 'importance in the digestive process of herbivorous animals, in whose stomachs and intestines it is very common.'

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[1] *Journal of Chemical Society,* 1891, p. 81.

Bacillus ulna.—This species is closely allied to *B. subtilis.* It measures 10 × 2 μ; and occurs singly, in chains, but it does not form leptothrix. It gives rise to spores which measure 2·8 μ × 1 μ. This microbe is found in rotting eggs. On the surface of bouillon it forms thick colonies which ultimately unite, giving rise to a pellicle. It is readily cultivated on sterilised egg-albumin.

Bacillus of Symptomatic Anthrax.—This microbe is the cause of the infectious disease known as quarter-evil, rauschbrand, charbon symptomatique, etc. The disease affects cattle, giving rise to the formation of an irregular tumour in the subcutaneous and intermuscular tissues. There is high fever, and death generally occurs in about forty-eight hours. This motile microbe (3 to 5 μ × 0·5 to 0·6 μ) is found in the serous fluids, bile, tumours in this disease. It has been cultivated in fowl broth to which small quantities of glycerol and ferrous sulphate have been added. It also grows on blood serum, nutrient gelatine, and vegetable albumin. As the microbe is anaërobic, it must be cultivated in an atmosphere devoid of free oxygen. It is best cultivated at the temperature of the body. Spore-formation takes place at the ends of the cells.

MM. Arloing, Cornevin, and Thomas[1] have shown that the virus is capable of giving immunity to animals inoculated with it. The following are the chief facts observed by them: (*a.*) Injection of a very small quantity of the virus into the loose connective tissue of any part of the body produces a temporary illness, and protects the animals. (*b.*) Injection of a moderate quantity into the scanty connective tissue of the tail produces a slight affection, and confers immunity. Very large doses, however, may cause death. A moderate quantity injected into the cellular tissue in other parts of the body causes death. (*c.*) Injection into the veins does not kill, but confers immunity, and the same result follows injection into the respiratory tract. (*d.*) Cultivation does not deprive the microbe of its virulence, but heating the spores to 85° C. for six hours destroys their virulence.

On page 117 of their book (*loc. cit.*), MM. Arloing, Cornevin, and Thomas state that the following substances destroy or do not destroy the virulence of this microbe:—

Do not destroy the virulence.	Destroy the virulence.
Alcohol (90 °/o).	Salicylic acid (0·1 °/o).
Glycerol.	Carbolic acid (2 °/o).
Sulphate of quinine (10 °/o).	Boric acid (20 °/o).
Hydrogen peroxide.	Sodium salicylate (20 °/o).
Sodium hyposulphite.	Potassium permanganate (5 °/o).
Ammonia.	Mercuric chloride (0·1 °/o).
Tannic acid (20 °/o).	Silver nitrate (0·1 °/o).

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[1] *Du Charbon Bactérien* (1883).

Bacillus ianthinus.—This motile microbe was first found in water, and differs from *B. violaceus* (also found in water) by not liquefying gelatine. It occurs singly, and in threads. On nutrient gelatine, agar-agar, and potatoes it produces white spots, which rapidly become violet. The pigment, which is soluble in alcohol, is only developed in the presence of air.

Bacillus violaceus.—This bacillus is also found in water. It grows as small round colonies on gelatine plates. These are first white, but rapidly assume a violet colour. It also grows on agar-agar, blood serum, and potatoes; giving rise, on each of these media, to the same pigment. The microbe is a motile rod about four times as long as broad, with rounded ends, and often contains spores.

Bacillus cyanogenus.—This microbe measures 2·5 to 3·5 μ × 0·4 μ; and occurs in chains and zooglœa. Spore-formation is also present. In test-tube cultivations, it gives rise to a white head, while the surrounding gelatine becomes blue or dark brown. In alkaline milk, it gives rise to a slate-coloured pigment; while in acid or sour milk, a beautiful blue pigment is developed (in fact, it is called 'the microbe of blue milk'). On agar-agar, it forms a brown pigment. It also grows on potatoes, boiled rice, starch-paste, etc.; and the colouring matter which is formed varies with the nourishing medium. These pigments are freely developed at from 15° to 18° C., but at 37° C. no colour is formed at all.

Bacillus erythrosporus.—This bacillus was found in putrefying albuminous fluids, potable water, etc. It occurs singly and as leptothrix. On gelatine-plates, white colonies are formed. The outer zones of these colonies are of a yellowish-green colour. On potatoes, this microbe forms brown patches which do not spread. It produces dirty-red spores.

Bacillus œdematis maligni.—This microbe, obtained from soil, is a pathogenic microbe. Mice, rats, cats, etc., inoculated with a pure cultivation of this bacillus, die in a few hours. It measures from 3 to 5 $\mu \times 1$ μ, and has rounded ends. It occurs singly, in chains, and leptothrix (straight or curved); spores are formed; and the microbe is anaërobic. It grows well on the surface of a neutral solution of Liebig's extract of meat at 36° to 38° C., or in nutrient agar-agar; but air must be excluded from the cultivation tubes or flasks.[1] For some recent work concerning the microbe of malignant œdema, see Dr. Klein's paper in *Centralblatt für Bakteriologie und Parasitenkunde,* Band x. (1891), p. 186.

Bacillus of rhinoscleroma.—A microbe found in the tissues of patients suffering from rhinoscleroma—a disease which gives rises to tumours on the lips, and nasal and pharyngo-laryngeal regions. The bacillus measures from 1·5 to 3 $\mu \times 0·5$ to 0·8 μ; it has rounded ends, produces spores, and surrounds itself with an elongated capsule (Fig. 33, 11). This microbe is readily stained with a solution of methyl violet.

Bacillus of Indigo Fermentation.—This microbe is morphologically similar to the bacillus of rhinoscleroma; and it has been proved by Alvarez[1] to be the cause of the indigo fermentation and the production of indigo-blue. Indigo-blue or indigotin is the product of several plants belonging to the *Indigofera* and other genera. It does not exist in these plants ready-formed, but is produced by the decomposition of a glucoside ($C_{26}H_{31}NO_{17}$) called indican. By the action of Alvarez's bacillus, indican yields indigo-blue (C_8H_5NO) and indiglucin ($C_6H_{10}O_6$):—

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[1] See Dr. Griffiths' *Researches on Micro-Organisms,* p. 235.

$$C_{26}H_{31}NO_{17} + 2\ H_2O = C_8H_5NO + 3\ C_6H_{10}O_6.$$

The bacillus of indigo fermentation has been shown to possess pathogenic properties, and occasions in animals a transient local inflammation, or death, with visceral congestion and fibrinous exudations.

Bacillus pyocyaneus.—This microbe is a very minute, short, thin rod; and it is said to produce spores. It occurs in chains of twos or threes, or collected into irregular masses; and it has been isolated from pus of those cases in which the wounds exhibit a greenish-blue colour. According to Dr. Gessard,[2] *B. pyocyaneus* produces a greenish pigment of a definite composition, which has been called 'pyocyanin.' Pyocyanin can be extracted from pus by means of chloroform. Dr. J. Kunz[3] has grown this microbe on nutrient gelatine kept for three or four days at the ordinary temperature, and then for seven days at 35° C. It liquefies the gelatine, which shows a green fluorescence, and has the specific smell of blue pus. Kunz extracted from the liquefied gelatine pyocyanin and pyoxanthose, but the liquid still showed a green fluorescence due to a distinct colouring matter, which is only soluble in water and alcohol, and is not destroyed by boiling. Concentrated solutions of this colouring matter transmit red and green light only, but dilute solutions have no absorptive power. According to Kunz, pyocyanin contains nitrogen and sulphur. The green pigment which is formed when this bacillus is grown on nutrient gelatine is most probably produced by the oxidizing action of the air on a chromogen which is formed by the bacillus, as the pigment is not contained in the bacillary cells. In gelatine solutions, the green colour disappears gradually at the ordinary temperature in ten or fifteen weeks, giving place to a dark reddish-brown colour, and the reaction becomes strongly alkaline. *B. pyocyaneus* grows in milk, and produces a yellowish-green solution, which becomes intensely green when ammonia is added.

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[1] *Comptes Rendus de l'Académie des Sciences*, tome 105.

[2] *De la Pyocyanine et de son Microbe*, 1882.

[3] *Monatshefte für Chemie*, Bd. ix. p. 361.

The chemistry of the microbian pigments is a subject which has been very little investigated; but these pigments are undoubtedly products formed from the decomposition of albuminoids by the agency of microbes.

Bacillus septicæmiæ (rabbit).—This microbe, which is pointed at both ends, measures 1·4 × 0·7 μ. It occurs singly and in chains; and it grows in bouillon, nutrient gelatine, and blood serum. On gelatine-plates, it produces 'dot-like colonies, and in test-tubes little spherical masses in the needle track, and a layer on the free surface.' This bacillus was isolated by Koch from putrid meat infusion and river-water. It is innocuous to guinea-pigs and white rats; but rabbits, mice, and birds are very susceptible to the attacks of this microbe.

Bacillus septicæmiæ (*mouse*).—This non-motile bacillus was isolated from garden soil and putrefying fluids. It measures 1 × 0·1 μ, and occurs singly, in pairs, and chains of four or more. It grows on gelatine-plates, in the deeper layers of the medium, as delicate white clouds. In test-tube cultivations, it produces delicate branching growths along the track of the needle. On agar-agar, lemon-yellow colonies are formed. This bacillus kills house-mice in forty to sixty hours; but field-mice have an immunity.

Bacillus septicæmiæ (*man*).—In human septicæmia, Klein[1] found in the blood-vessels of the lymphatic glands certain bacilli which form continuous masses in the capillaries and small veins. These bacilli measure 1 to 2·5 μ × 0·3 to 0·5 μ, and occur singly or in short chains.

Bacillus diphtheriæ vitulorum.—This microbe measures 2·5 to 3·6 × 0·5 μ, and was described by Löffler as occurring in the diphtheria of calves. Mice inoculated from a calf died with all the characteristic symptoms of the disease. The microbe has not been artificially cultivated.

Bacillus diphtheriæ columbarum.—This bacillus was isolated from the false membrane of the diphtheria of pigeons. It is a short rod with rounded ends, and occurs in irregular masses. On the surface of gelatine, it forms light yellow films, while in the deeper layers of that medium white nodules are formed. This bacillus destroys pigeons, sparrows, mice, and rabbits; but fowls, guinea-pigs, rats, and dogs have an immunity.

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[1] *Micro-Organisms and Disease*, p. 120 (3d ed.).

Bacillus of Diphtheria of Rabbits.—This microbe measures 3 to 4 $\mu \times 1 \mu$; it has rounded ends, and occurs singly, in pairs, or in long chains. On gelatine-plates it forms grey colonies, which become brown. It was isolated during growth in 'the diphtheritic processes of the intestine;' and causes (in rabbits) an inflammatory exudation in the alimentary canal.

Bacillus cavicida.—This microbe was discovered, by Dr. Brieger, in fæces and putrefying fluids. The rods are very small, and they form colonies composed of white concentric rings on gelatine plates. On potatoes they give rise to dirty yellow masses. They are fatal to guinea-pigs, but not to mice and rabbits.

Bacillus pyogenes fœtidus. — A microbe with rounded ends, and measuring $1{\cdot}45 \times 0{\cdot}58 \mu$, was isolated from putrid pus. It occurs in pairs or chains, and is motile and produces spores. On the surface of gelatine and agar-agar it forms greyish films, and on potatoes a shining brown growth is developed. From all these media a strong putrid odour emanates, but no smell is given off when the microbe is cultivated in milk. It is fatal to mice and guinea-pigs.

Bacillus of Swine Erysipelas.—This bacillus has been obtained from the blood of pigs which have died of the disease. It measures 1·1 μ × 0·2 μ. In test-tube cultivations it produces a cloudy growth in the track of the needle. It is fatal to mice, pigeons, and rabbits, as well as pigs.

Bacillus of Ulcerative Stomatitis in the Calf.—Drs. A. Lingard and E. Batt[1] discovered certain bacilli in ulcerations on the tongue and mucous membrane of the mouth of calves (Fig. 33, 13). They measure 4 to 8 μ × 1 μ, and occur singly and as leptothrix forms, the filaments of which are either straight or more or less curved. They contain spores; and when injected into a mouse or rabbit they produce a fatal result. These bacilli are best stained by immersion in a mixture of methylene blue and magenta.

Bacillus of Swine Plague.—This microbe[2] measures 2 to 3 μ in length, and produces spores. It was observed in the organs of pigs which had died of swine fever, or swine plague. It is readily cultivated in broth and hydrocele fluid at temperatures ranging between 30° and 42° C. A drop of either of these cultures inoculated into pigs, rabbits, and mice produce the disease, with multiplication of the bacilli; and 'the animals die with a characteristic swelling to the spleen, coagulative necrosis of tracts of the liver tissue, and inflammation of the lungs.' Pigs inoculated with artificial cultures of the microbe are protected against a fatal attack.

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[1] *Lancet*, 1883.

[2] See Klein's *Micro-Organisms and Disease*, pp. 131-136.

Bacillus putrificus coli.—It was first isolated from fæces, and measures about 3 μ in length. On gelatine it has an opalescent appearance, but finally becomes a yellow colour. It is motile microbe, which occurs in long or short threads. Spore-formation has been observed.

Bacillus epidermidis.—It was discovered in the fragments of epidermis taken from between the toes. This microbe measures from 2·8 to 3 μ in length, and 0·3 μ in breadth; it forms spores from 1·2 to 1·5 μ in length, and 0·3 to 0·4 μ in breadth. It grows only sparsely on nutrient gelatine and agar-agar. On potatoes it forms a characteristic superficial skin.

Bacillus of Nitrous Fermentation.—Dr. P. F. Frankland[1] has recently isolated from soil a bacillus which converts ammonia into nitrites. This microbe will be described under the heading of 'the microbes of the soil.'

Bacillus megaterium.—This microbe was discovered by the late Dr. De Bary on boiled cabbage. The rods are motile, and measure 10 μ × 2·5 μ. They occur singly and in chains, and grow on gelatine and agar-agar, forming a whitish layer. On potatoes at 20° C. yellowish-white dots are formed. *B. megaterium* is an aërobic microbe, and produces spores.

Leptothrix buccalis.—This microbe occurs in the slime of the teeth, on the epithelium of the mouth, etc.; in other words, it is one of the microbes of the mouth. It occurs as isolated bacilli or threads, generally arranged in bundles (Fig. 38), which may be interwoven with one another. Each thread is divided into short rods, from 1 to 1·2 μ broad, and from 2 to 10 μ long. This microbe is believed to be connected with dental caries.

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[1] *Philosophical Transactions of the Royal Society*, 1890, p. 107.

Leptothrix innominata.—This microbe is usually found on the soft white matter which is deposited on the teeth. The threads are from 0·5 to 0·8 μ in breadth.

Leptothrix parasitica.—The threads are slender, not articulated, loosely felted, and for the most part curled. They measure from 100 to 140 μ in length, and about 1 μ in breadth, and occur both in still and running water. This bacillus (Fig. 33, 22) is best cultivated on infusions of rotting algæ and animal substances. This microbe is believed by Zopf and others to give rise to micrococci, bacteria, etc.; in other words, it is a pleomorphic form, but Zopf's observations were not made after exact methods.

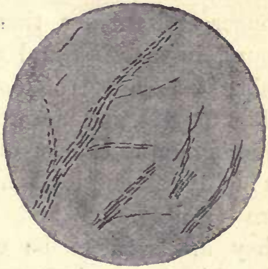

FIG. 38. LEPTOTHRIX BUCCALIS.

Beggiatoa roseo-persicina.—This is the 'peach-coloured bacterium' of Ray Lankester,[1] and is really a sulpho-chromogenic bacillus. It occurs 'on the surface of marshes, or on water in which algæ are rotting, and sometimes these bacilli are in such quantity that the whole marshes and ponds may be coloured blood-red by them.' *B. roseo-persicina* contains dark-coloured sulphur granules, the dark colour being due to the pigment—bacterio-purpurin—formed by the microbe. This pigment is insoluble in water, alcohol, etc., and when examined spectroscopically it shows a strong absorption band in the yellow, a weaker band in the green and blue, and a darkening in the more refrangible half of the spectrum.

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[1] *Quarterly Journal of Microscopical Science*, vol. xiii. p. 408.

Beggiatoa alba.—This bacillus occurs as threads without distinct articulations. The threads are longer and thicker than leptothrix, and they are found in marshes and sulphur springs. The cells (about 3·5 μ broad) of *B. alba* contain sulphur granules (Fig. 33, 20), and, according to Cohn and Cramer,[1] these granules consist of crystalline sulphur, which is highly refractive. When these crystalline granules are disintegrated and examined microscopically, they are seen to be composed of a number of rhombic (octahedral) crystals. A variety (*B. alba marina*) of this microbe forms a delicate white gelatinous membrane on decaying animals and algæ in a marine aquarium.

Beggiatoa nivea.—The threads of this bacillus are very slender, indistinctly jointed, and form undulated woolly tufts of milky-white colour. *B. nivea* occurs in sulphur springs.

Beggiatoa mirabilis.—The microbe occurs in sea-water, forming a white gelatinous scum on decomposing algæ, etc. The threads are very thick, motile, bent and curled in various ways, and they have rounded ends. They are distinctly articulated (16 μ broad), and contain sulphur granules.

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[1] *Beiträge zur Biologie der Pflänzen*, vol. i.

Besides the four last-mentioned microbes there are *B. leptomitiformis, B. arachnoidea,* and *B. pellucida,* each of which contains sulphur granules. These microbes play an important part in the elimination of sulphur and the disengagement of sulphuretted hydrogen. The sulphogenic or sulphur-forming microbes are found in certain waters, and many of the natural sulphurous waters are due to the action of these microbes on alkaline sulphates and organic matter present in such water. The decomposition of calcium sulphate by sulphogenic microbes may be represented by the following equations:—

(α) $3\ CaSO_4 + H_2O = S_2 + H_2S + 3\ CaO + 5\ O_2$.
(β) $2\ CaSO_4 = S_2 + 2\ CaO + 3\ O_2$.

Sulphogenic microbes are also capable of decomposing animal and vegetal albumin with the liberation of sulphur.

Bacillus septicus.—This microbe occurs in soil, putrid blood, and other fluids. Its breadth varies from 4 to 10 μ, and its length depends on the number of elements contained in a row: the shortest are about 4 μ. It is a non-motile bacillus, capable of forming leptothrix and spores.

Bacillus of conjunctivitis.—This bacillus was obtained from the conjunctival sac in cases of conjunctivitis. It grows on agar-agar plates as pearly dots, and in bouillon. The latter medium is the best for the cultivation of this microbe. It measures from 1 to 2 μ in length, and 0·25 μ in breadth.

Bacillus figurans.—This microbe was first described by Crookshank,[1] and occurs in soil and in the atmosphere. It has rounded ends, and forms spores. On the oblique surface of agar-agar it forms a feather-like growth. On gelatine plates *B. figurans* causes 'a cloudy growth, spreading from various points.' When 'cultivated in nutrient gelatine this bacillus forms on the surface visible windings, from which fine filaments grow down into the gelatine. They spread out also in almost parallel lines transversely from the needle track.'

Bacillus Hansenii.—The rods measure 2·8 to 6 μ × 0·6 to 8 μ, and are best cultivated on steamed potatoes, where they form a deep yellow layer, which has the odour of amylic alcohol. Ultimately the yellow layer dries, and changes to a brown colour, at the same time forming spores (1·7 μ × 1·1 μ). This bacillus occurs on bouillon, wine, and malt infusions, which have been kept at about 32° C.

VIBRIONES.

These microbes are rod-shaped, but not straight; they are more or less wavy, and they are motile.

Vibrio serpens.—This vibrio measures from 11 to 25 μ long, and from 0·8 to 1·1 μ in breadth. It occurs in various infusions.

Vibrio rugula.—The rods measure from 6 to 16 μ in length, and about 0·5 to 2·5 μ in breadth. They are curved or S-shaped, and bear a flagellum at each end. They swarm when causing decomposition in vegetable infusions. According to Prazmowski, *Vibrio rugula* develops a spore at one end of the cell.

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[1] *Lancet*, 1885.

SPIRILLA.

Spirillum tyrogenum.—This spirillum measures about 0·8 to 1·5 μ in length. On gelatine plates (see Fig. 24) it forms colonies of a greenish-brown colour. In test-tubes the gelatine becomes liquid along the needle-track, while on agar-agar a pale yellow layer develops. This microbe, which is non-pathogenic, was isolated, by Deneke, from old cheese. *S. tyrogenum* is capable of withstanding a temperature of —18° C. for several days.[1]

Spirillum Finkleri.—The rods are curved, and they are larger and thicker than the *Spirillum choleræ Asiaticæ.* On gelatine-plates they grow rapidly, forming small white dots with a brownish tinge; and the gelatine is liquefied very rapidly. The fluid (from the liquefaction) becomes completely turbid, whereas in *S. choleræ Asiaticæ* the upper part remains clear. In gelatine tube cultivations, liquefaction occurs in the form of a funnel-shaped tube, and the fluid becomes turbid. On agar-agar and potatoes white films or layers are formed. *S. Finkleri* was discovered in the dejecta of cases of cholera nostras, and it was said to be identical with the *Spirillum* of Asiatic cholera; but it is quite distinct.

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[1] Griffiths in *Proc. Roy. Soc., Edinb.*, vol. xvii. p. 263; and *Researches on Micro-Organisms*, p. 176.

Spirillum Obermeieri.—This microbe is the cause of relapsing fever, and was first discovered by Obermeier[1] in the blood of patients suffering from the disease. Carter[2] reproduced the disease in monkeys, in whose blood and organs the spirilla were found in great numbers. This microbe (16 to 40 μ long), which is motile, exhibits spiral forms, and, according to Albrecht,[3] produces spores. *S. Obermeieri* (Fig. 33, 7) has been artificially cultivated by Koch.[4] The microbe only occurs during the relapses, and is absent during the non-febrile intervals.

Spirillum tenue.—This spirillum measures from 4 to 15 μ in length, and about 2·25 μ in breadth. It usually occurs in various infusions, in which it moves about with great rapidity. It occurs in swarms or united in a zooglœa.

Spirillum undula.—It measures from 8 to 12 μ in length, and from 1·1 to 1·4 μ in breadth (Fig. 33, 5). There is a flagellum at each end, and the microbe is actively motile; although at times it forms a zooglœa. It occurs in bog-water and various infusions.

Spirillum volutans.—This microbe occurs in marsh water and various infusions. It measures from 20 to 30 μ in length, and 1·5 to 2 μ in breadth (Fig. 33, 6). The protoplasm contains a number of dark granules, and there is a flagellum at each end.

Spirillum sanguineum.—This was observed by

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[1] *Centralblatt für Med. Wissensch.*, 1873.

[2] *Lancet*, vol. i. p. 84, and p. 662.

[3] *St. Petersb. Med. Woch.*, 1879.

[4] *Deutsche Med. Woch.*, vol. xix.

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Cohn and Warming in pond-water. It is said to be morphologically identical with *Spirillum volutans.* The cells contain numerous red bodies and many sulphur granules. According to Saville Kent,[1] this microbe is not identical with Ehrenberg's *Ophidomonas sanguinea*: the latter being a true monad.

Spirillum concentricum.—This microbe was discovered by Kitasato in putrefactive blood. It grows rapidly on gelatine-plates, giving rise to greyish-white round colonies, each of which has concentric markings. It does not liquefy the gelatine, and is non-pathogenic.

Besides the above-mentioned spirilla, there are the following, which occur in brackish and sea water: *S. violaceum*, *S. Rosenbergii*, *S. attenuatum*, etc.; but the reader is referred to the works of Warming for an account of these microbes.

SPIROCHÆTÆ.

Spirochœta plicatilis.—This microbe is of extraordinary length—110 to 225 μ (Fig. 33, 19). It occurs in stagnant water. The threads are arranged in wavy lines.

Spirochœta gigantea.—The threads are blunt at both ends. It occurs in sea-water.

YEAST-FUNGI.

These fungi are not microbes (*i.e.* they are not *Schizomycetes*), but belong to an altogether different order—the *Saccharomycetes.* They multiply chiefly by gemmation or budding, but they can also produce spores, especially when they are deprived of nourishment. These organisms occur widely distributed in air, soil, and water, and they are the cause of the alcoholic fermentation.

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[1] *Manual of the Infusoria*, p. 244.

Saccharomyces cerevisiæ.—This organism is sometimes termed *Torula cerevisiæ,* and is the true ferment of beers. The cells are round or oval (8 to 9 μ long), and are either isolated or united in small colonies. The spore-forming cells (when isolated) measure from 11 to 14 μ long; and the spores measure from 4 to 5 μ in diameter. This organism occurs in beers brewed by both the high or low systems of fermentation. Fig. 39, 1 and 2, represent the beer-ferments. There are two races of this species, high (1) and low (2) yeasts. The cells of high yeast are slightly larger and more round than those of low yeast. Low yeast never rises to the surface of the fermenting wort, which is kept at a temperature varying from 4 to 5° C. This low fermentation is a slow process, occupying about fourteen days. The low fermentation gives rise to 'Lager' or 'Bavarian' beer. High yeast rises to the surface as the fermentation proceeds, and the wort is kept at a temperature varying from 16° to 20° C. The fermentation is rapid, and rarely occupies more than a few hours or so. The high and low yeasts are not different species. Both high and low yeasts secrete a soluble enzyme which converts maltose and sucrose into invert sugars (dextrose and levulose):—

$$\underset{\text{[maltose]}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{[dextrose]}}{C_6H_{12}O_6} + \underset{\text{[levulose]}}{C_6H_{12}O_6}.$$

Saccharomyces minor.—This organism (Fig. 39, 3) consists of a spherical cell measuring 6 μ in diameter. It occurs in chains of six or nine cells. The spore-forming cells each measure from 7 to 8·5 μ in diameter, and contain from 2 to 4 spores, each having a diameter of 3·5 μ. Hansen and Engel state that this yeast is the cause of fermentation in bread.

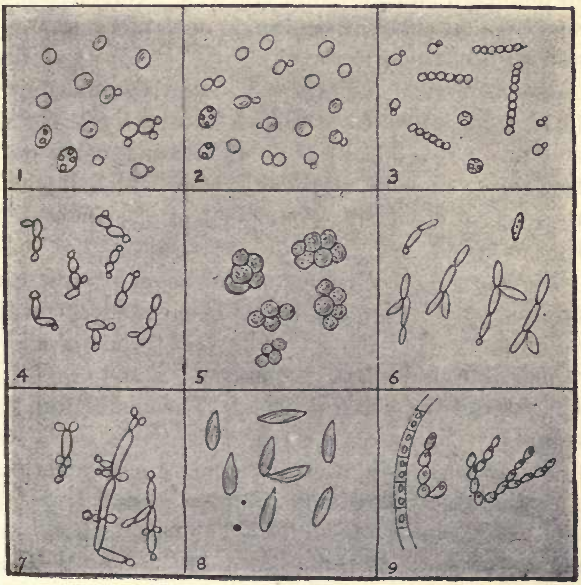

FIG. 39. YEAST-FUNGI.

Saccharomyces ellipsoideus.—The cells are elliptical (Fig. 39, 4), mostly 6 μ long, and are isolated or united in little branched colonies. Two to four spores are found in a mother cell. It is a low yeast when grown in beer wort; but it is really a species of wine ferment, which produces the spontaneous fermentation in must.

Saccharomyces conglomeratus.—The cells are almost round (Fig. 39, 5), measuring from 5 to 6 μ in diameter, and united in clusters. This organism occurs in wine at the beginning of the fermentation, and on decaying grapes.

Saccharomyces exiguus.—The cells are conical (5 μ × 2·5 μ), and are united in slightly branched colonies (Fig. 39, 6). Spore-forming cells each contain from two to three spores, which lie in a row. This organism occurs in the after-fermentation of beer.

Saccharomyces Pastorianus.—The cells are oval or elongated (Fig. 39, 7). 'The colonies consist of primary club-shaped links (18 to 22 μ long), which build lateral, secondary, round or oval daughter-cells (5 to 6 μ long).' The spores number from two to four. This organism occurs in the after-fermentation of wine, fruit-wines, and fermenting beer. It is very common in the air.

Saccharomyces apiculatus.—The cells are lemon-shaped (Fig. 39, 8) and from 6 to 8 μ long × from 2 to 3 μ broad, and sometimes slightly elongated. Gemmation occurs only at the pointed ends. Spore formation is unknown. It occurs in fermented wine, in spontaneous fermentations of all kinds of fruits, and in certain kinds of beer. It is a low yeast, giving rise to a feeble alcoholic fermentation, and it does not invert sucrose. When mixed with *S.*

cerivisiæ it retards the action of the true beer ferment.[1]

Saccharomyces mycoderma.—The cells are oval, elliptical, or cylindrical (Fig. 39, 9), measuring about 7 μ long and about 2 μ thick. They are united in richly-branched colonies; and the cells are often elongated, so as to resemble a hyphal filament. This organism forms the scum on the surface of beer, wine, sauerkraut, and fruit-juices. It has nothing to do with the alcoholic fermentation; and is not identical with *Bacterium aceti* (*Mycoderma aceti*), which is the *microbe* of the acetic fermentation in wines and beers.

Saccharomyces vini.—This organism is the true wine-producing ferment, for it is the cause of the alcoholic fermentation of grape-juice. Its cells are elliptical, slightly smaller than those of *S. cerevisiæ.* It forms spores, and is very common in the atmosphere.[2]

It should be borne in mind that fermentation is not a chemical, but a vital process; for the researches of Pasteur and others have shown that every fermentation has its specific ferment; in all fermentations in which the presence of an organised ferment has been ascertained the ferment is necessary.

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[1] See Martinand and Reitsch's paper in the *Comptes Rendus*, t. 112 (1891).

[2] For further information concerning the yeasts see Jörgensen's *Micro-Organisms of Fermentation;* Pasteur's *Etudes sur la Bière*, *Etudes sur la Vin;* Engel's *Les Fermentes Alcooliques;* and the papers of Hansen.

CHAPTER VI

INFECTIOUS DISEASES AND MICROBES, ETC.

'THE study of disease-germs by the new and accurate methods of bacteriology has led to a clearer and better understanding of the manner in which, at any rate, some of the infectious diseases spread. While it was understood previous to the identification of their precise cause that some spread directly from individual to individual (*e.g.* small-pox, scarlet fever, diphtheria), others were known to be capable of being conveyed from one individual to another indirectly, *i.e.* through adhering to dust, or being conveyed by water, milk, or by food-stuffs (*e.g.* cholera, typhoid fever). But we are now in a position to define and demonstrate more accurately the mode in which infection can and does take place in many of the infectious diseases. By these means we have learned to recognise that the popular distinction between strictly contagious and strictly infectious diseases—the former comprising those diseases which spread, as it were, only by contact with a diseased individual, while in the latter diseases no direct contact is required in order to produce infection, the disease being conveyed to distant points by the instrumentality of air, water, or food—is only to a very small extent correct. Take, for instance, a disease like diphtheria, which was formerly considered a good example of a strictly contagious disorder; we know now that diphtheria, like typhoid fever or scarlet fever, can be, and, as a matter of fact is, often conveyed from an infected source to great distances by the instrumentality of milk. In malignant anthrax, another disease in which the contagium is conveyable by direct contact, *e.g.* in the case of an abrasion or wound on the skin coming in contact with the blood of an animal dead of anthrax, we know that the spores of the anthrax bacilli can be, and, in many instances are, conveyed to an animal or a human being by the air, water, or food. The bacilli of tubercle, finding entrance through a superficial wound in the skin or mucous membrane, or through ingestion of food, or through the air, can in a susceptible human being or an animal produce tuberculosis either locally or generally. The difference as regards mode of spread between different diseases resolves itself merely into the question, Which is, under natural conditions, the most common mode of entry of the disease-germ into the new host? In one set of cases, *e.g.* typhoid fever, cholera, the portal by which the disease-germ generally enters is the alimentary canal; in another set an abrasion or wound of the skin is the portal, as in hydrophobia, tetanus, and septicæmia; in another set the respiratory organs, or perhaps the alimentary canal, or both, are the paths of entrance of the disease-germ, as in small-pox, relapsing fever, malarial fever; and in a still further set the portal is just as often the respiratory tract as the alimentary canal, or a wound of the skin, as in anthrax, tuberculosis. But this does not mean that the virus is necessarily limited to one particular portal, or that it must be directly conveyed from its source to the individual that it is to invade. All this depends on the fact whether or not the microbe has the power to retain its vitality and virulence outside the animal or human body.'[1]

It must be borne in mind that not all the diseases described in the present chapter can, at the present time, be termed true microbian diseases; yet with the progress of science, and by following the lines already laid down, we have not the slightest doubt that in time the microbes of all the infectious diseases will be discovered and cultivated.

YELLOW FEVER.

Micrococci (0·6 to 0·7 μ diam.) have been found in the kidney, spleen, and liver during the course of yellow fever. They form rosaries and masses, which greatly distend the blood-vessels and give rise to hæmorrhages. The yellow-fever microbe is termed *Micrococcus amaril* by Dr. Domingos Freire. This microbe grows on gelatine, and reproduces the disease in rabbits and guinea-pigs. If, however, the microbe is cultivated in gelatine for six generations it loses the greater part of its virulence, and when this attenuated virus is introduced into the body by inoculation, it produces a mild type of yellow fever, and confers immunity against the fatal type of the disease. From 1883 to 1890 Freire[1] has inoculated 10,881 persons in Brazil with cultures of *M. amaril.* The mortality of those so vaccinated was 0·4 per cent., although the patients lived in districts infected with yellow fever, whilst the death-rate of the uninoculated during the same period was from 30 to 40 per cent.

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[1] From a lecture delivered at the Royal Institution, London (February 20, 1891), by Dr. E. Klein, F.R.S.

Yellow fever is distributed (within certain areas) by moist winds and human intercourse. Water and the soil have nothing to do with the spread of the disease, although it is a disease which clings to the ground, hence one of the reasons of its endemic nature. It is always prevalent in the plains near the sea-coast, and along the courses of the great rivers. Heat (21° C.) and a certain saturation of the atmosphere are essential conditions for an epidemic of yellow fever. Frost puts an end to an epidemic at once, and storms, heavy rains, or cold weather check its progress.

HYDROPHOBIA.

Hydrophobia or rabies is a canine disease, which is communicated by a bite, and the inoculation of man and other animals by the saliva. The exact nature of the microbe of this disease is not yet known. According to Pasteur,[2] Fol,[3] Babès,[4] and

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[1] *Comptes Rendus*, 1889 and 1891.

[2] *Comptes Rendus*, 1884.

[3] *Ibid.*, 1885, p. 1276; *Les Microbes*, 1885, p. 41.

[4] *Les Bactéries*, 1890, p. 550.

Dowdeswell,[1] the microbe appears to be a micrococcus, and it has been observed in microscopical sections of the spinal cord of animals dead of rabies. Dr. Fol's preparations were made by hardening the spinal cord or brain by immersion, directly after death, in a solution containing 2·5 grammes of potassium bichromate, and 1 gramme of copper sulphate in 100 cc. of water. The piece of tissue is divided so as to be able to take up Weigert's solution of hæmatoxylin; then placed in absolute alcohol, imbedded in paraffin, and cut into sections not more than $\frac{1}{200}$ mm. in thickness. The sections are finally decolorised by a solution containing 2·5 grammes of potassium ferrocyanide, 2 grammes of borax in 100 cc. of water. In these sections, Fol found small micrococci ($0{\cdot}2\ \mu$ in diam.) in the lymph spaces of the neuroglia, and between the

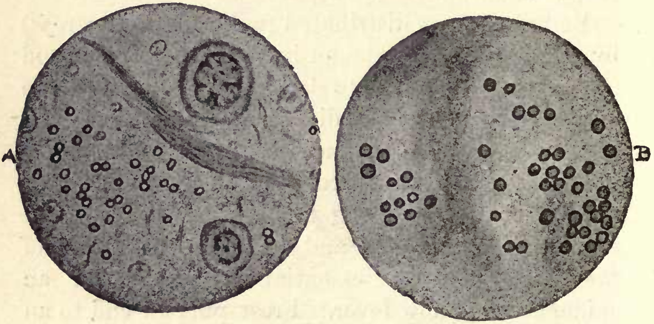

FIG. 40. MICROCOCCI IN HYDROPHOBIA.
A, In cerebral matter (*after Fol*). B, In human saliva.
× 1000

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[1] *Journ. Roy. Microscop. Society*, 1886; and *Lancet*, 1886.

axis cylinder and its medullary sheath. This microbe (Fig. 40A) occurs in groups and as diplococci, but never in chains. According to Fol, if a cultivation (in bouillon) be made of part of the brain, there is a deposit which, on inoculation into healthy animals, produces all the features of rabies. If, however, the cultivation be more than six days old there are no marked toxic effects. Fol says that nothing can be distinctly made out by merely reducing the nervous tissue of a rabid animal to a pulp and examining it microscopically, as recommended by Gibier.

Babès states that he has found micrococci in the brain and spinal cord of rabid animals. These measure from 0·6 to 0·8 μ in diameter, *i.e.* from three to four times as great as the microbe described by Fol. These micrococci are stained *in situ* by Löffler's alkaline methylene blue solution. They are cultivated on blood serum or agar-agar (at 37° C.), and on bouillon made with the brain of a rabbit. The micrococci grow slowly and give rise to grey spots. 'A pure culture of the second, or even of the third generation, when inoculated into animals occasionally produces hydrophobia, but in most cases the cultures have no pathogenic properties, and it must, therefore, be concluded that the microbe has either lost its virulence or that it is not the actual cause of the disease.'

The late Mr. G. F. Dowdeswell observed a microbe, measuring about the same diameter as Babès' micrococcus, in the central canal of the

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[1] *Comptes Rendus*, 1883, p. 1701; 1884, pp. 55 and 531.

spinal cord and the medulla oblongata of dogs dead of rabies.

The author has also observed a micrococcus (Fig. 40B) in the saliva of a woman suffering from hydrophobia.[1] The micrococcus, which is deeply stained by methylene blue, measures from 0·6 to 0·8 μ in diameter. This microbe does not occur in healthy human saliva.

We cannot say that the microbe of rabies has been isolated with anything like success, for the above investigations do not fulfil Koch's canons (see Chapter I.) to ascertain the pathogenic nature of the microbe or microbes in question. It is probable that the virus of rabies will not develop in the absence of a living pabulum, and in all probability it is not possessed of powers of active resistance to those injurious influences which act upon it when exposed to the air, etc. In fact, the virus of rabies cannot survive the drying, changes of temperature, etc., it necessarily undergoes when scattered over the ground, as we often see happen by the slobbering of a rabid animal.

The saliva of rabid animals does not contain a ptomaïne, for when it is diluted with a small quantity of sterilised distilled water, and then heated to 90° C. for a few hours, the saliva loses its virulent power. This proves that no alkaloid was present, because it would not have been destroyed on the application of heat.[2] Besides, M. Nocard dialysed the pure saliva of rabid animals, and proved that its solid constituents were always virulent, and reproduced the disease when injected into healthy animals, while the fluid portion, similarly injected, remained inactive. If an alkaloid or ptomaïne had been present it would have been found in the fluid portion, and would have given rise to toxic effects when injected into the system. Although a ptomaïne has not been discovered in the saliva of rabid animals, Dr. Anrep[1] isolated a poisonous ptomaïne from the brain and medulla oblongata of rabbits suffering from rabies. This ptomaïne reproduced all the characteristic symptoms of the disease, and it is stated that a gradual habituation of the animal to small doses of the ptomaïne produced a certain degree of immunity.

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[1] The saliva was kindly sent to the author by Dr. T. M. Dolan, of Halifax.

[2] Griffiths' *Researches on Micro-Organisms*, p. 193.

Rabies is not a disease of the blood, for the supposed microbe is not found in the blood system, and when the blood of a rabid animal is injected into animals it does not reproduce the disease. In fact, the virus is located in the nervous system, especially the medulla oblongata.

The period of incubation of rabies is usually not less than from four to six weeks, and sometimes longer. 'At the end of this incubation period the wound, first of all, becomes slightly uncomfortable; there is itching, and the heat becomes almost intolerable, especially as this is usually accompanied by a sharp stinging pain; the patient becomes feverish and very thirsty; the face is pallid and has a peculiar anxious expression, the muscles of the face being drawn and restless, and gradually this expression amounts to one of actual terror or horror. On the second or third day the patient becomes much more excited, is restless in every sense of the word, and a very peculiar feature is that he has a characteristic habit of giving a suspicious side-glance as though constantly looking out for some hidden danger; then as the fever advances a rambling delirium supervenes; the thirst increases, but along with this there is great difficulty in swallowing—especially fluids—and after making one or two attempts to swallow, the very sight of water suggests such horrors that, thirsty as the patient is, he is anxious to avoid it. Then muscular tremors are noted; these become more and more marked, and violent spasms are easily stimulated, as in tetanus. A sharp sound, a touch, a bright light, or even a breath of air, may give rise to violent muscular convulsions, and eventually the patient is slowly suffocated as in tetanus' (Woodhead). Such are some of the torturing symptoms of hydrophobia. But it may be stated that the symptoms are varied, depending upon the nature of the region in the nervous system—encephalon or spinal cord—where the virus locates itself. The virus is found in every part of the encephalon. Although the saliva of rabid animals is virulent, it is not used by Pasteur in his prophylactic treatment, the reason being that, as saliva contains various microbes, it may give rise to septic poisoning, etc., as well as rabies. Therefore Pasteur has recourse to the central nervous system, where the virus is obtained in a pure state. This pure virus is continually being inoculated on the surface of the brain of healthy animals; the object of this is to keep up the supply of the virus in question. The virus can be intensified or modified by passing it through various animals. For instance, by passing it from the dog to the monkey, and subsequently from monkey to monkey, the virus grows weaker at each passage, until its virulence entirely disappears. Successive passages from rabbit to rabbit, and from guinea-pig to guinea-pig, increase the virulence of rabies virus. The intensified virus comes to a fixed maximum in the rabbit. If now transferred to the dog it remains intensified, and shows itself to be much more virulent than the virus of ordinary street rabies. So great is this acquired virulence, that the intensified virus injected into the blood-system of a dog unfailingly gives rise to mortal madness. These facts suggested to Pasteur that, by keeping a set of attenuated viruses of different strength, some not mortal, he could preserve the animal economy against the ill effects of more active ones, and these latter against the effects of mortal ones.

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[1] *British Medical Journal*, 1889, p. 319.

The sets of attenuated viruses are not obtained by the passage of the virus through different animals, for the method now in use at the Pasteur Institute consists in suspending portions (a few centimetres in length) of the spinal cords of inoculated rabbits in a dry atmosphere (*i.e.* the marrows are desiccated in sterilised bottles of one litre capacity by means of caustic potash). By this method the virulent power gradually diminishes, and finally disappears. By using attenuated viruses of varying intensities (prepared by desiccation), Pasteur has successfully treated numberless animals and human beings which are now refractory to rabies.

To prepare the inoculating fluid a mad dog is killed, and the brain and medulla oblongata are carefully removed with sterilised instruments, etc. Very small pieces of the medulla oblongata and of the central canal are then placed in a sterilised glass. They are triturated with a glass rod, and when reduced to a fine jelly-like mass sterilised veal bouillon is added in quantity to about half a table-spoonful. This dilute dog-virus is used for inoculating a rabbit on the surface of the brain. A full-grown living rabbit is placed upon a dissecting board, flat on its abdomen, and its four limbs secured by strings to pegs driven in the wood (Fig. 41). After this the animal is placed under the influence of chloroform. The hair is cut away, and an incision, one inch long, is made from a point midway between the eyes. The operator cuts down to the skull, which is then trepanned (Fig. 41*a*), and a little circular disc of bone is removed, as far as possible without injuring the external membrane of the brain. At this point the operator takes a hypoder-

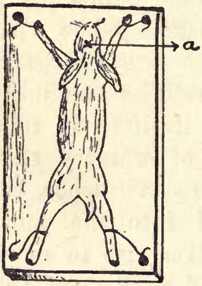

Fig. 41. Trepanning a Rabbit.

mic syringe (see Fig. 20), filled with the diluted dog-virus, and inserts it under the dura mater, injecting two drops of the virus. The disc of bone is then replaced, and the skin flaps are sewed together by means of two or three sutures. 'A pad of cotton wadding, carefully purified by heat, is used to dry the skin, after which a little of the same wadding is used as a dressing; this dressing is kept in position by a free application of flexile collodin, the two together forming an air-proof shield, through which no microbes from the external air can make their way to the wound, which, as a rule, heals up most perfectly in less than a couple of days.'

After death the brain and medulla oblongata are removed, and a dilute virus is prepared from them, as in the case of the dog-virus. This is injected beneath the dura mater of a second rabbit, the operation being repeated in fresh rabbits until the shortest incubation period has been reached. This incubation period of seven days' duration is reached by the fiftieth passage, the rabbit taking ill on the seventh day, and dying on the tenth day or later, is the one used for human inoculations as well as for the purpose of perpetuating the disease in other rabbits. By dealing with a sufficiently large number of animals it is possible to have a rabbit dying every day, and thus also to put one spinal cord in a desiccating bottle every day. By the fourteenth day there will be a set of fourteen marrows undergoing the desiccation. These marrows vary in virulence. The marrow of one day's desiccation is the most virulent, and the virulence of the other marrows decreases gradually until the fourteenth day of desiccation, when a minimum is reached. At the Pasteur Institute, the marrows of more than fourteen days are thrown away as being inert and useless.

A person having been bitten by a mad dog is first injected[1] with the weakest virus, and on each successive day or so with gradually stronger viruses until the more powerful or most powerful virus is used. After this treatment the patient very rarely dies of rabies. During the years 1886-9 no less than 7893 patients were treated at the Pasteur Institute, and out of this number there were 53 deaths, which represents a mortality of 0·67 per cent. But since 1889 the mortality has been reduced to 0·2 per cent., due, no doubt, to the better skill in the application of the treatment.[2]

It may be stated in passing that at the Pasteur Institute, Paris, there are two rabbits inoculated, and, consequently, also two dying (of rabies) every day, 'for fear if one alone were used it might die from accident, and the series be interrupted. Practically one animal is found to be quite sufficient, and the second one is only inoculated for prudence' sake.'

'The medulla or cord of a rabbit in which the incubation has been seven days, when injected intracranially into a dog, develops rabies in the latter animal in about twelve days. The nervous matter of this dog, inoculated back by the same process into rabbits, at once reproduces the malady after an incubation of seven days, and thus the series is recovered.'

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[1] In the hypochondria (*i.e.* certain abdominal regions).

[2] Concerning the interesting statistics of the Pasteur Institutes of St. Petersburg, Odessa, Moscow, Warsaw, Charkow, Turin, Bucharest, Naples, and Havannah, the reader is referred to the latest edition of Cornil and Babès' book—*Les Bactéries* (1890).

Pasteur's treatment is prophylactic and not curative, for it is powerless against the disease when the first symptoms have once made their appearance. Hence the necessity of early treatment.

The mode of action of the Pasteurian inoculations has been explained by the two following theories: (1) Metschnikoff[1] states that the white blood-corpuscles (phagocytes) absorb and digest the living microbes, and their power of absorption for microbes is trained and increased by the progressively stronger inoculations, so that finally the virus deposited by the rabid animal can also be absorbed and destroyed. The whole process is carried out, therefore, in the lymphatic system. (2) Woodhead and Wood believe that the treatment consists essentially in causing the tissues to acquire a tolerance before the microbe has had time to develop. 'The tissue cells are acted upon by increasingly active virus, each step of which acclimatises the cells for the next stronger virus, until at length when the virus formed by the microbes introduced at the time of the bite comes to exert its action, the tissues have been so far altered or acclimatised that they can continue their work undisturbed in its presence, and, treating the microbes themselves as foreign bodies, destroy them. When the cells are *suddenly* attacked by a *strong dose* of the poison of this virus they are so paralysed that the microbes can continue to carry on their poison-manufacturing process without let or hindrance; but when the cells are gradually though rapidly, accustomed to the presence of the poison by the exhibition of constantly-increasing doses, they can carry on their scavenging work even in its presence, and the microbes are destroyed, possibly even before they can exert their full poison-manufacturing powers. Some such explanation as this would account for the interference with the course of the disease even after the patient has been bitten. The microbe is localised, it takes some time to form its poisonous products, and whilst this is going on the whole of the nervous and other tissues are being gradually acclimatised by the direct application of small quantities of the poison artificially introduced.'[1]

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[1] *Fortschrift der Medicin*, 1885.

In concluding our remarks concerning rabies, it may be stated that the rabid marrows can be preserved for several months in pure and neutral glycerine. Hence the use of this fluid for preserving the marrows (for inoculation against rabies) during their transit from France to foreign countries.

For further information the reader is referred to the undermentioned books and papers on the subject.[2]

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[1] Woodhead's *Bacteria and their Products*, p. 327.

[2] Pasteur in *Comptes Rendus*, 1881-86; Dolan's *Hydrophobia: M. Pasteur and his Methods*; Gamaleia in *Annales de l'Institut*

ERYSIPELAS.

This disease is due to the *Micrococcus erysipelatosus* (0·4 μ diam.) which abounds in the lymphatic vessels of the skin at the margin of an erysipelatous zone. This microbe, which is smaller than *M. vacciniæ*, occurs singly and in chains, as well as zoogloea. The microbe grows on nutrient gelatine, agar-agar, and solid blood-serum, as a whitish film on the surface of the nourishing medium. Orth[1] and Fehleisen[2] have both cultivated the microbe artificially, and reproduced the disease in rabbits. But Fehleisen went a step further and reproduced the disease in man by inoculating three patients with pure cultivations of the microbe. 'These inoculations were justifiable because they were undertaken with a view to cure certain tumours. Thus one case of lupus, one case of cancer,[3] one case of sarcoma, were considerably affected, and to the good of the patients.' In the human subject typical erysipelas was produced in fifteen to sixty hours after inoculation.

Pasteur, 1887; Reyes in *Gac. Méd. Mexico*, 1889, p. 344; Dolan in *Provincial Medical Journal*, 1890, p. 137; Zagari in *Giornale Internazionale delle Scienze Mediche*, 1890; Hime in *Lancet*, 1886, p. 184; Griffiths' *Researches on Micro-Organisms*, p. 323.

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[1] *Archiv für Experim. Pathol.*, 1874.

[2] *Die Aetiologie des Erysipels*, 1883.

[3] If cancer is due to Scheuerlein's Cancer bacillus, it is probable that the *M. erysipelatosus* is antagonistic to its growth.

PUERPERAL FEVER.

According to Heiberg,[1] micrococci have been found in the form of chains and zooglœa in all organs affected in this disease. Heiberg's micrococcus has not yet been artificially cultivated, consequently we cannot say that the microbe is the real cause of this highly infectious disease.[2] The infectiousness of puerperal fever is now well established, although the microbe or microbes which give rise to the different symptoms classed under the name of puerperal fever have not been isolated. Certain poisonous ptomaïnes have been isolated by Bourget from the viscera of a woman who died of puerperal fever, and subsequently he proved the existence of the same ptomaïnes in the urine of patients suffering from the same disease. Pasteur and others are convinced that with the possible exception of cases where, by the presence either of internal or external abscesses, the body before confinement contains microbes, the antiseptic treatment ought to be infallible in preventing puerperal fever from declaring itself. It may be stated that the introduction of the antiseptic and aseptic methods has produced not only a remarkable diminution of mortality, but also of the morbidity or illness incident to the puerperal state.

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[1] *Die Puerperalen und Pyämiochen Processe.*

[2] In 1889 a midwife carried the contagion to five different women, all of whom died of the disease (*The Echo*, Sept. 17, 889).

Prof. I. Giglioli[1] gives the following statistics concerning the patients in the Maternity Hospital at Copenhagen between the years 1850 and 1874 (the antiseptic method being introduced in the year 1870):—

From 1850 to 1864	the mortality was	41·6	per 1000.
„ 1865 „ 1870	„ „	19·6	„
„ 1870 „ 1874	„ „	11·4	„

And in Naples the mortality during the years 1875-78 was 0·12 per 1000 patients.

Dr. W. O. Priestley gives in his paper, which was read before the Congress of Hygiene,[2] an interesting table showing the maternal deaths in six lying-in hospitals since the introduction of antiseptic and aseptic methods. With these he contrasted the figures of M. Le Fort before the era of antiseptics:—

MORTALITY IN MATERNITY HOSPITALS FROM ALL CAUSES IN VARIOUS COUNTRIES OF EUROPE (LE FORT).

Before the introduction of Antiseptics.

	Deliveries.	Deaths.	Per 1000.
Total, .	888,312	30,394	34·21

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[1] *Fermenti e Microbi*, p. 157.

[2] Held in London during September 1891.

After the introduction of Antiseptics.

	Date.	Deliveries.	Deaths.	Deaths which would have occurred on basis of Le Fort's figures.
Vienna, . . .	1881-5	15,070	106	516
Dresden, . . .	1883-7	5,508	57	188
Russia, . . .	1886-9	76,646	290	2,622
New York, . .	1884-6	1,919	15	66
Boston, . . .	1883-6	1,233	27	42
London (General Lying-in Hospital),	1886-9	2,585	16	88
Total, . .		102,961	511	3,522

The number of lives saved out of the 102,961 deliveries since the introduction of antiseptics is the following:—

Expected deaths on Le Fort's basis, .	3522
Actual deaths, . . .	511[1]
Saving, . .	3011

From the above figures it will be seen that while according to M. Le Fort, the maternal deaths in European lying-in hospitals were 34·21 per 1000 under the old *régime*, the mortality is now reduced to somewhat less than 5 per 1000. This computation, put in another way, indicates that if the former rate of mortality had been maintained 3522 maternal deaths might have been expected, whereas the actual deaths were only 511. In other words, 3011 lives of mothers were saved as the result of new and purely scientific methods of treatment.[1]

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[1] 4·363 per 1000.

INFLUENZA.

This is a very different disease from the catarrhal affections known by the same name. It is really an acute specific disease running a definite course like scarlatina or measles; but very little is known of the cause or nature of this ubiquitous disease which has attacked humanity in its own violent fashion at short intervals from probably the earliest ages. The history of the recorded epidemics of *La Grippe* is marvellously complete for centuries. Every country and every climate in the world is subject to it, yet it appears to find a permanent home nowhere as a constant or endemic resident, but to disappear from the face of the earth for a series of years. It is, however, probable that the microbe of this disease has some undiscovered endemic source.

The symptoms of epidemic influenza follow precisely the type of the other infective fevers, and preserve a remarkable uniformity and individuality in successive epidemics. Sir Morell Mackenzie[2] believes that the disease is due to 'a specific poison of some kind which gains access to the body, and, having an elective affinity for the nervous system, wreaks its spite principally or entirely thereon. In some cases it seizes on that part of it which governs the machinery of respiration, in others on that which presides over the digestive functions; in others again it seems, as it were, to run up and down the nervous key-board, jarring the delicate mechanism, and stirring up disorder and pain in different parts of the body, with what almost seems malicious caprice.' Therefore, according to Mackenzie, the supposed microbe resides in, or acts on, the nervous tissues of the body.

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[1] For further information on the subject of puerperal fever see Flessinger's paper in *Gaz. Méd. de Paris*, 1889, p. 313; and Widal's *Etude sur l'Infection Puerpérale* (1889).

[2] *Fortnightly Review*, June 1891.

There are many reasons for thinking that the contagium of influenza is borne through the air by winds rather than by human intercourse. One reason for thinking so is that it does not appear to travel along the lines of human communications, and, as is seen in the infection of ships at sea, is capable of making considerable leaps. Dr. Parsons, on the other hand, believes that the epidemic is propagated mainly by human intercourse, though not in every case necessarily from a person suffering from the disease.

Concerning the germ of influenza, Klebs thought that he had discovered this in certain *Flagellata* found in the plasma or corpuscles of the blood during the febrile stage, but no cultivations were made. Gluber found a micrococcus (in pairs) in the blood; and Fränkel noticed the same microbe in the sputum of a patient suffering from influenza. This microbe may have been *Micrococcus pneumoniæ,* as pneumonia frequently follows an attack of the disease. Ribbert thinks that *Micrococcus pyogenes* is invariably present, and is the actual cause of influenza; but Besser has shown that it is common in healthy men at least during the epidemic. In 1884 Seifert found a micrococcus in the sputa of influenza patients and of no others.

Although the microbe of influenza has not yet been isolated, there is little doubt that influenza is a microbian disease; for its constancy of type, the mode of its transmission, its independence of climatic and seasonal conditions, all suggest that its cause is 'specific'—*i.e.* having the properties of growth and multiplication which belong to a living thing.[1]

PNEUMONIA.

In this disease large numbers of micrococci are present in the lungs.

The microbe (*Micrococcus pneumoniæ*, Fig. 33, 17) was discovered by Friedländer,[2] and occurs in the sputa of pneumonic patients, either singly, as diplococci, short chains, and zooglœa. Sometimes the microbes are free, while at other times they are encysted in the lymphatic cells. They are oval, encapsulated microbes, and have been cultivated in blood-serum, peptonised gelatine, bouillon, and on steamed potatoes.

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[1] See Sisley's *Epidemic Influenza* (1891); Brodie's paper in *Nature*, July 23, 1891; Parson's *Report on Influenza to the Local Government Board* (1891); the Hon. R. Russel's pamphlet, *The Spread of Influenza* (1891); Cantani's *L'Influenza* (1890); Talamon's *La Grippe et les Microbes* (1890). See also the Appendix.

[2] *Virchow's Archiv*, vol. lxxxvii.

Griffini and Cambria[1] observed the same micrococci in the blood of pneumonic patients. Salvioli and Zäslein[2] cultivated these microbes, derived from the same source, in bouillon at 37° to 39° C.; and when injected into mice and rabbits they gave rise to pneumonia. Giles[3] found the same microbes in many cases of pneumonia in India; and pure cultivations, when injected into the subcutaneous tissues of rabbits, produced the disease. Those researches have been confirmed by Afanassiew.

When the artificially-cultivated microbe is inoculated in the tissue of the lungs it produces in animals all the characteristic symptoms of pneumonia; the lungs become red, solid, and enlarged, and pieces of them sink in water. In pneumonia the blood is considerably altered, for the hæmatin, globulin, and the salts are greatly reduced.

According to Emmerich, the growth of the micrococcus of pleuro-pneumonia on peptonised gelatine is similar to the one derived from human pneumonic sputum; and when this microbe is injected into rabbits it produces typical pneumonia. Nolen and Poels[5] injected pure cultivations of the microbe of human pneumonia into cattle, and produced pleuro-pneumonia with all its characteristic symptoms. However, it may be mentioned that

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[1] *Centralblatt für d. Med. Wissensch.*, 1883.

[2] *Ibid.*, 1883.

[3] *British Medical Journal*, 1883.

[4] *Comptes Rendus de la Société de Biologie* (Paris), t. 5.

[5] *Centralblatt für d. Med. Wissensch.*, 1884.

Dr. Klein[1] does not accept these statements without reservation.

Professor Brieger[2] has shown that when *M. pneumoniæ* is grown in solutions of glucose or sucrose, acetic acid is formed along with ethyl alcohol and formic acid. The same products are formed when the microbe is grown in solutions of creatine and calcium lactate.

Dr. P. F. Frankland[3] has recently investigated the action of the same microbe on various carbohydrates, with the following results:—

(*a*) *Micrococcus pneumoniæ* sets up a fermentive process in solutions of dextrose, sucrose, lactose, maltose, raffinose, dextrin, and mannitol.

(*b*) It does not ferment solutions of dulcitol or glycerol, and has thus the power, like the *Bacillus ethaceticus* (see p. 155), of distinguishing between the isomers, mannitol, and dulcitol.

(*c*) The fermentation of mannitol is represented by the following equation:—

$$6C_6H_{14}O_6 + H_2O = 9C_2H_5HO + 4CH_3COOH + 10CO_2 + 8H_2.$$

In other words, the above equation represents the quantitative decomposition of mannitol into alcohol, acetic acid, carbon dioxide, and hydrogen.

It would be interesting to ascertain whether acetic acid and alcohol are formed in human milk during an attack of pneumonia; for it may be stated that the lactose is reduced from 43·6 to 30·2 parts per 1000.

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[1] *Micro-Organisms and Disease*, p. 77 (3d. ed.).

[2] *Zeit. Physiol. Chem.*, vol. viii. p. 306; and vol. ix. p. 1.

[3] *Journal of Chemical Society*, 1891, p. 253.

SCARLATINA.

This disease is the result of the action of the *Micrococcus scarlatinæ,* which has been found in the blood, organs, the exudations and tissues of the ulcerated throat, and in the desquamating epidemic cells of this disease. *M. scarlatinæ* has also been observed in the urine of patients suffering from scarlatina; and this fluid contains a ptomaïne represented by the formula $C_5H_{12}NO_4$. The same ptomaïne has also been extracted from pure cultivations of *Micrococcus scarlatinæ* in peptonised gelatine. In fact, the microbe forms this ptomaïne from the medium in which it lives.[1]

M. scarlatinæ (0.5 μ diam.) occurs singly, as diplococci, in chains, and in masses (Fig. 33, 9); and it grows on the surface of nutrient gelatine, as well as in the depth of that medium. It also grows on agar-agar and in beef bouillon. On nutrient gelatine with slanting surface this microbe forms greyish, circular, flat discs, which ultimately form a grey film. Gelatine tubes inoculated by stabbing show some characteristic features. After twenty-four hours' incubation the surface of the stab appears sunk, and the depression thus formed increases in breadth and depth during the next two or three days, so that by that time there is a distinct funnel-shaped depression indicating the upper end of the channel of inoculation marked as a white streak. Then, commencing at the bottom of the funnel, the gelatine becomes liquefied, and this liquefaction gradually extends in breadth, and always in depth, along the line of the growth. The liquefied part of the gelatine is clear, and at the bottom of it is a whitish precipitate.

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[1] See Dr. A. B. Griffiths' paper in *Comptes Rendus de l'Académie des Sciences,* vol. cxiii. (Nov. 9. 1891), p. 656.

In stab-cultivations, using agar-agar or solid blood serum as the medium, white dots make their appearance in the streak; but these are of a brownish colour where thickly packed together. On the surface of agar-agar or solid blood serum a continuous film is formed.

In alkaline bouillon the growth forms whitish, fluffy, or loose masses at the bottom of the tube. In milk *M. scarlatinæ* grows fairly well, and turns the milk at first thick, then quite solid; sometimes this occurs after two or three days' incubation at 37° C., sometimes a little later.

Drs. Klein and Power have proved that a certain eruptive disease of the teats and udders of cows is capable of communicating scarlatina to human beings through the medium of the milk derived from such cows.

In certain extensive outbreaks of scarlatina at Hendon, Wimbledon, etc., Dr. Klein found *Micrococcus scarlatinæ* in the blood of scarlatina patients both during life and after death; and he found the same microbe in the tissues and organs of persons dead of scarlatina. These outbreaks of scarlatina were traced by Power and others to the milk-supply from certain farms where the cows were suffering from what is now known as cow-scarlatina. In both human and bovine scarlatina the same microbe

(*M. scarlatinæ*) is always present in the tissues, organs, and blood; and from both sources subcultures of the microbe, when inoculated into healthy cows, produce the disease. For instance, when pure subcultures of the microbe were inoculated into calves and cows, the microbe was found in the spleen, kidneys, teats, udders, lung, skin, etc. Fig. 42 represents a section through the skin of the nostril of a calf that had been experimentally infected with *M. scarlatinæ* derived from a human source. In this figure it will be observed that the microbe is present in large numbers.

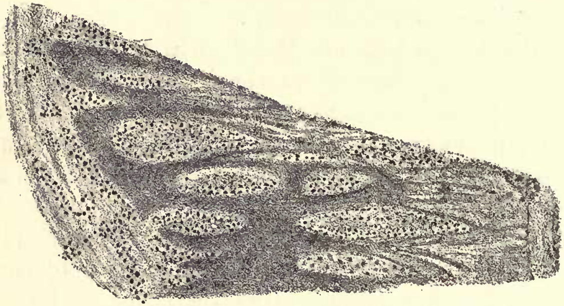

FIG. 42. SECTION THROUGH SKIN OF THE NOSTRIL OF A CALF THAT HAD BEEN EXPERIMENTALLY INFECTED WITH M. SCARLATINÆ DERIVED FROM A HUMAN SOURCE (*Klein*).

In fact, Dr. Klein's important researches on the relationship existing between the cow-disease, already alluded to, and human scarlatina may be summarised as follows:—

(*a*) The disease in man and in the cow alike is characterised by closely similar anatomical features (*b*) From the diseased tissues and organs of man and cow alike the same microbe can be separated, and artificial subcultures be made from it.

(*c*) These subcultures, no matter whether established from man or cow, have the property, when inoculated into calves, of producing in them every manifestation of what is known as the Hendon cow-disease, except the sores or ulcers on the teats and udders—no doubt for the reason that the milk apparatus is not yet developed in calves.

(*d*) Subcultures of the microbe made from human scarlatina and inoculated into recently calved cows produced in them, along with other manifestations of the Hendon cow-disease, the characteristic ulcers on the teats—ulcers identical in character with those observed at the Hendon farm.

(*e*) The subcultures, established either from the human or the cow disease, have an identical property of producing in various rodents a disease similar in its pathological manifestations to the Hendon disease of cows and to scarlatina in the human subject.

(*f*) Calves fed on subcultures established from human scarlatina obtain the Hendon disease.

(*g*) Children fed on milk from cows suffering from the Hendon disease obtain scarlatina.

Bearing on the same subject, it may be mentioned that in the parish of St. George's, London, five persons were attacked with scarlatina on the same day (in October 1886). They had used a *cheap* brand of condensed milk; and in this milk Dr. Klein proved (both by cultivation and inoculation) the presence of *Micrococcus scarlatinæ*.

These investigations prove that cows suffer from scarlatina; that the specific microbe circulating in the blood of the diseased animals contaminates the milk; and that such milk conveys the disease to human beings. The disease has also been directly communicated to man by inoculation with the virus from the ulcers on the teats and udders. In a particular case, recorded by Dr. J. Cameron,[1] a man received the virus of scarlatina into a recent scratch upon his forefinger while milking a diseased cow.

As both human beings and cows are liable to be attacked with scarlatina, and as the milk of the latter (when diseased) is capable of producing an extensive outbreak of the disease in human beings, it is advisable that milk should be boiled before use. This destroys any microbes which may be present.[2]

LEPROSY.

The microbe of this disease is Hensen's *Bacillus lepræ.* It measures from 4 to 6 μ long and about 1 μ wide (Fig. 33, 2), and it occurs in masses within the large leprosy-cells of the nodules of the skin and organs, as well as of the mucous membrane of the mouth, palate, and larynx. Two types of leprosy are described—the anæsthetic and tubercular varieties; the first variety is more frequently seen in the tropics, the latter in temperate climates. In the anæsthetic variety the bacillus is present in the interstitial tissue of the nerves. *B. lepræ* is sometimes motile and produces spores. It grows on blood serum and alkaline infusions of meat-extract; and Damsch[1] has produced the disease in cats by inoculating them with leprous tissues. The microbe is absent in the blood of lepers; therefore it probably spreads by the lymphatic vessels.

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[1] *Transactions of Epidemiological Society*, 1885-6.

[2] For full details of Klein's researches see the *Reports of Medical Officer to Local Government Board,* 1885-6; 1886-7; 1887-8; 1887-9.

Hansen's discovery[2] of *B. lepræ* has since been confirmed by Neisser,[3] Cornil,[4] Babès,[5] Hillis,[6] Stevens,[7] Thin,[8] Rake,[9] Köbner,[10] Bordoni-Uffreduzzi,[11] and Gianturso;[12] and during the present Leprosy Commission in India, Drs. Rake, Buckmaster, Thomson, and Kanthack have also succeeded in rearing *B. lepræ*[13] on blood serum; but growths of this microbe are difficult to obtain. Bordoni-Uffreduzzi obtained 'growths from the marrow of a bone in which there were a number of free leprosy bacilli; these appeared on serum (to which a quantity of glycerine had been added) that was maintained at a temperature of 37° C. These he described as delicate, thin, slightly yellow films with irregular borders. On glycerine agar-agar they are said to have developed as small, grey, rounded, isolated points, usually at the end of ten days or a fortnight; secondary cultivations, however, made their appearance at the end of forty-eight hours, and after the first few cultivations the microbe could be grown on serum or on ordinary gelatine and agar-agar, but much more slowly than when glycerine had been added.'

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[1] *Virchow's Archiv*, vol. xcii.

[2] *Ibid.* vol. lxxix.

[3] *Ibid.* vol. lxxxiv.

[4] *Union Médicale*, 1881.

[5] *Archives der Physiologie*, 1883.

[6] *Transactions of Pathological Society*, 1883.

[7] *British Medical Journal*, 1885.

[8] *Med.-Chir. Transactions*, vol. lxix.

[9] *Transactions of Pathol. Soc.*, 1887.

[10] *Virchow's Archiv*, vol. lxxx.

[11] *Zeitschrift für Hygiene*, vol. iii. p. 178.

[12] *Centralblatt für Bakteriologie und Parasitenkunde*, vol. ii. p. 701.

[13] Concerning the differences between the leprosy and tubercle bacilli, see Slater's paper in *Quart. Journ. Micros. Science*, 1891.

Leprosy, or elephantiasis grecorum, is a specific disease, characterised by the slow development of nodular growths in connection with the skin, mucous membranes, and nerves, and by the supervention of anæsthesia, paralysis, and a tendency to ulcerative destruction and gangrene.

Although prevalent in the Middle Ages, leprosy is very rare in Europe at the present day, being confined to isolated areas on the shores of Spain, Portugal, Sweden, Norway, Iceland, and in Italy, Roumania, Hungary, and Greece, where it is still endemic. It is, however, common in Egypt, Morocco, Cape Colony, Madagascar, Southern Asia (including Japan), Brazil, United States of Colombia, Guiana, Argentina, New Zealand, and in certain islands of the Pacific Ocean (especially Hawaii).

In the United States of Colombia leprosy first made an appearance in 1646, and was introduced into that country from Spain. It seems to have spread slowly but surely throughout a great part of the country during the succeeding two hundred years; but since 1870 the increase in the number of cases has been much more rapid, and within that period the disease has spread to districts where it was previously unknown, until now almost every district in Colombia is more or less infected. According to a medical authority residing in Bogota, it is stated that one-tenth of the inhabitants of Santander and Boyaca are lepers. As the population of these two states is about 1,000,000, this estimate would give 100,000 lepers in that portion of Colombia alone. Another authority states that there are only 30,000 lepers in the two states previously mentioned; but whichever figure is correct, it shows that a large percentage of the inhabitants are suffering from this fell disease. Marriages constantly take place between non-lepers and lepers, and children are born of these unions; but they generally develop the disease in a few years. The lepers also marry among themselves, and their children are almost always lepers. Very little is done in the way of isolation, consequently leprosy is bound to spread more and more throughout Colombia unless some great effort is made to arrest its progress. It is the universal opinion all over Colombia that leprosy is both contagious and hereditary; but it is probable that the system requires to be predisposed by bad food, unsuitable climate, dirty and confined lodging, exposure to chills and damp, etc., before leprosy can be contracted by contagion. There is no doubt that the absence of hygienic appliances and personal cleanliness aid its development immensely.[1]

So far as is at present known, there is no cure for

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[1] See the *British Consular Report from Bogota*, 1891.

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leprosy;[1] but no doubt, with growing experience, leprous vaccine will soon be discovered; and it is even possible that, with the experience already gained, such a result may at once be obtained (Pasteur).

SYPHILIS.

Syphilis is a specific disease; and, 'after the local introduction of the syphilitic poison, some ten to fifty days elapse before the true Hunterian chancre first appears, but at the same time indurated buboes or glands may be detected in the groins. In a few weeks the blood becomes tainted by the peculiar virus, and this interfering with the nutrition of the blood capillaries and tissues, produces a series of morbid phenomena, divided by syphilographers into secondary and tertiary, the term primary being retained for the manifestations due to local inoculation. Leaving no tissue untouched,[2] syphilis is well known also for the variety of its manifestations and for its propensity to attack parts of the body often respected by other forms of skin disease and blood poisoning. A proneness to leave behind much dusky, copper-coloured staining of the skin, whilst its inflammatory eruptions scarcely cause itching, are features of diagnostic interest.'

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[1] It is stated that leprosy has been cured by the 'Mattei remedies' *(Report of the St. Joseph's Asylum at Mangalore,* 1891), but these 'remedies' have been proved to be quack preparations, etc., by the medical profession.

[2] Hence the reason that Byron called this disease—'the great:'—

> 'I said the small-pox has gone out of late;
> Perhaps it may be follow'd by the great.'
>
> (*Don Juan*, c. I., v. 130.)

The *Bacillus of syphilis* was discovered by Dr. S. Lustgarten[1] in the nucleated cells of various syphilitic products, *e.g.* 'in the discharge of the primary lesion and in hereditary affections of tertiary gummata.' He never found the microbe free between the tissue elements, but always enclosed in cells. Nevertheless, it may be stated that Eve and Lingard[2] isolated a bacillus from the *blood*, as well as from the diseased tissues in syphilis, which they cultivated in artificial media.

Lustgarten's bacillus measures from 3 to 4 μ long and 0.8 μ wide (Fig. 33, 21); it has a swelling at each end. It is believed that this microbe produces spores, and, according to Lustgarten, it is the virus of syphilis. Doutrelepont, De Giacomi, and Schütz have confirmed Lustgarten's observations.

TETANUS.

Tetanus or lockjaw is an infectious disease caused by the *Bacillus of tetanus*, which inhabits certain soils; for it was proved by Nicolaier[3] that soil obtained from streets and fields,[4] when inoculated into mice, rabbits, and guinea-pigs, gave rise to the characteristic symptoms of tetanus. The microbe of this disease forms spores. 'These spores gaining access to an abrasion or wound of the skin in man or animals, are capable of germinating there and multiplying, and of producing a chemical poison, which is absorbed into the system, and sets up the acute complex nervous disorder called lockjaw.'

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[1] *Med. Jahrbücher der K. K. Gesellsch. d. Aerzte* (Vienna), 1885.

[2] *Lancet*, 1886, p. 680.

[3] *Dissertation* (Göttingen), 1885.

[4] Soils obtained from cultivated gardens and from woods do not give rise to tetanus.

The tetanus bacillus (1.2 μ long) produces spores only at one end (Fig. 43), and in the spore-bearing condition is known as the drum-stick-shaped bacillus. It is motile and anaërobic, growing on gelatine-plates (containing glucose) in an atmosphere of hydrogen. In tubes containing blood serum or nutrient gelatine, it grows in the depth of the medium, forming a kind of cloud. The medium emits a fusty smell, which is characteristic of this microbe.

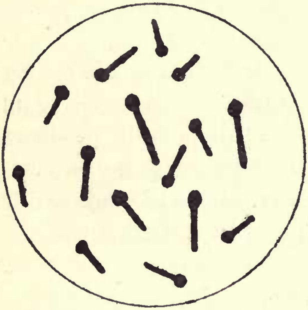

FIG. 43. THE TETANUS BACILLUS. (× 1000.)

In obtaining cultivations of the tetanus bacillus, other anaërobic microbes grow, and also produce spores. But Kitasato found that the tetanus bacillus produced spores earlier than the other bacilli present in tetanic pus. Consequently, he devised the following method for separating the tetanus bacillus from the other microbes :—As soon as spore-formation in the tetanus bacilli had commenced, the tubes (containing them) were heated for a considerable time at 80° C., with the result that all the bacilli were destroyed, but not the *spores* of the tetanus bacilli. These spores afterwards germinated (at 30° C.), and give rise to *pure* cultivations of the tetanus bacillus.

This microbe is localised at the actual point of inoculation (*i.e.* in the pus and the walls of the abscess), and is never present in the internal organs. The ptomaïne, which the tetanus bacillus gives rise to, is manufactured at the site at which it is actually introduced, and 'from this point it is absorbed into the body, and is carried to the special tissues on which it acts.'

Professor L. Brieger[1] has succeeded in isolating four ptomaïnes from pure cultivations of the tetanus bacillus. This first is tetanine ($C_{13}H_{30}N_2O_4$), which produces tetanus in animals; the second is tetanotoxine ($C_5H_{11}N$), which produces tremor and paralysis, followed by violent convulsions; the third is spasmotoxine (formula unknown), which produces tonic and clonic convulsions; and the fourth ptomaïne (which has not been named) causes tetanus, accompanied with a flow of saliva and tears.[2] Tetanine has also been extracted from the limb of a patient who had died from tetanus. Brieger looks upon the poisonous substance tetanotoxine as a toxalbumin; but he may have overlooked the possibility that this proteid may contain a ptomaïne closely bound to it, or in an isolated condition within its molecules.

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[1] *Virchow's Archiv*, vol. cxii. (1888), p. 549; vol. cxv. (1889), p. 484; *Berliner Klinische Wochenschrift*, 1888; and *Untersuchungen über Ptomaine*, 1886, p. 89.

[2] These tetanic ptomaïnes do not occur in the *urine* of patients suffering from tetanus.

As the tetanus bacillus is localised, there can be no doubt that tetanus is due to the above poisons (manufactured indirectly by the bacillus) producing effects after getting into the blood, by virtue of some selective action on certain parts of the motor nerve-centres.

The spores of the tetanus bacillus have an extremely wide distribution, being found in soils, etc., in various parts of the world. According to Bossano,[1] soils which contain much organic matter nearly always contain tetanus bacilli, 'and that latitude, climate, and special meteorological conditions have far less influence on their development than defective drainage, imperfect hygienic conditions, and the degree of cultivation of the soil.'

Dr. Kitasato[2] has recently shown how to produce immunity against tetanus, and he has cured animals suffering from this disease. Kitasato first renders an animal immune against tetanus, and then injects the blood serum of that animal into animals suffering from the disease. In order to render an animal immune or unsusceptible, the tetanus bacilli are first injected; this injection being followed by injections of iodine trichloride, which are repeated at intervals of twelve hours. After four days the animal, which under ordinary circumstances would have died from tetanus, is not only cured, but rendered immune against the disease. The blood serum of such an animal has been found in successive experiments on mice and rabbits to act as a complete cure. Kitasato's experiments prove (*a*) that the blood of rabbits which have been rendered unsusceptible to tetanus possesses properties destructive of the tetanus virus; (*b*) that these properties are to be observed also in extra-vascular blood and serum free from cells; (*c*) that these properties are of so permanent a nature that they are still manifested by such serum after it has been injected into other animals; consequently, by transfusion of such blood or serum, important therapeutic actions can be obtained; (*d*) that this power of destroying the tetanus poison is absent from the blood of such animals as are not immune against tetanus; and after such animals have been killed by the tetanus poison, it can be shown to be present in their blood and tissues.

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[1] *Comptes Rendus*, tome 107, p. 1172; and *Recherches Expérimentales sur l'Origine Microbienne du Tétanos* (1890).

[2] *Deutsche Medicinische Wochenschrift*, 1890, No. 49, *et seq.*

If animals (such as mice and rabbits) highly susceptible to tetanus are cured by this treatment, we have good reason to believe that it will also cure human beings, which are far less susceptible to the disease.

MALARIA.

The discovery of the *Bacillus malariæ* placed malaria among the acute specific diseases. Concerning the distribution of malaria, moisture and air have much to do with it, for the disease is more abundantly developed in wet than in dry years. Moisture in the soil is essential for the production of malaria, while clayey, loamy, and marshy soils favour its development. Professor C. Tommasi-Crudeli[1] states that the following conditions are necessary for the *Bacillus malariæ* to produce spores: (*a*) 'Une température de 20 degrés centigrades environ; (*b*) un degré modéré d'humidité permanente; (*c*) l'action directe de l'oxygène de l'air sur toutes les parties de la masse [that is, of the soil]. Il suffit que l'une de ces trois conditions fasse défaut, pour que le développement des sporules, et la multiplication du ferment malarique, soient arrétés.' In *marshy* districts, the larger the amount of organic matter present in a soil, the greater will be the prevalence of malaria. The disease is more prevalent the lower the level of the country, although in Central Africa a height of 2500 feet is not free from it. Both air and water may convey the disease, and there is little doubt that it finds an entrance into the system by means of air, potable water, and food.

Bacillus malariæ (2 to 7 μ long) gives rise to leptothrix filaments, and produces spores either at the ends or in the centre of the cell (Fig. 38, 18). This bacillus was found in the blood of malarial patients by Klebs and Tommasi-Crudeli,[2] and they also found it in the spleen, medulla, lymphatic glands, and venous blood of persons dead of malaria. On gelatine *B. malariæ* gives rise to a well-developed growth, and when a drop of the culture is inoculated in rabbits it reproduces malarial fever, with all its characteristic symptoms, the threads and spores of the bacilli being found in abundance both in the spleen and the marrow. This microbe grows also on albumin, urine, and other media in the presence of air, and at a temperature of about 20° C. *B. malariæ* was originally discovered in the soil of the Roman Campagna, and Antonio Ceci[1] obtained pure cultures of the microbe from this soil. When these pure cultures were inoculated in animals they produced malaria or intermittent fever.

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[1] *La Malaria de Rome et l'Ancien Drainage des Collines Romaines* (Paris), 1881; and *Atti della R. Accademia dei Lincei*, 1879.

[2] *Atti della R. Accademia dei Lincei*, 1879, 1880, and 1881; and *Archiv für Experimental Pathologie*, 1879.

Dr. B. Schiavuzzi[2] has confirmed Klebs and Tommasi-Crudeli's discovery of *Bacillus malariæ*, and that it is the real cause (directly or indirectly) of malaria. Cohn[3] has also verified the work of the Italian bacteriologists.

On the other hand, Laveran,[4] Richard,[5] Marchiafava and Celli,[6] Golgi,[7] Evans,[8] and others have discovered certain organisms allied to the *Flagellata* in the blood of patients suffering from malaria. These organisms have been called *Plasmodium malariæ*, and they are said to give rise to intermittent fever in man after intravenous injection. The blood corpuscles of a person so infected again contain the plasmodia; and it is further stated that these organisms alter the composition of the blood.

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[1] See Professor Giglioli's *Fermenti e Microbi*, p. 592.

[2] *Atti della R. Accademia dei Lincei*, 1886.

[3] *Beiträge zur Biologie der Pflanzen*, 1886, p. 245.

[4] *Comptes Rendus*, 1881-2.

[5] *Ibid.* 1882.

[6] *Annali di Agricoltura* (Roma), 1886, p. 4.

[7] *Archivio per le Scienze Mediche*, vol. x. (1886), p. 109.

[8] *Proceedings of Royal Society*, 1891.

In a paper read before the Accademia dei Lincei on May 2, 1886, Professor Tommasi-Crudeli[1] says that he does not accept the statement that the plasmodia found in the blood of malarial patients are the cause of malaria. In fact, he says 'la grande extensione dell' infezione malarica : le varie forme, ora lente e latenti, ora rapide e intense, nelle quali questa infezione si manifesta : la lunga persistenza, anche allo stato latente, della malaria in un terreno : son tutti forti argomenti contrari alla ipotesi che la malaria sia dovuta ad un parassita di natura animale ; e favorevoli all 'opinione che i germi malarici siano Schizomiceti, simili a quelli delle tuberculosi, e di altre persistenti infezioni.'

The alteration in the composition of the blood in patients suffering from malaria (previously alluded to) may be due to a soluble enzyme secreted by *B. malariæ* (Schiavuzzi), and certainly this is not improbable, for Dr. Lauder Brunton, F.R.S.[2] has shown that many microbes have the power of 'manufacturing a ferment suited to their needs.'

Bacillus malariæ is inhaled into the blood by way of the lungs, and perhaps it may enter through the stomach and skin also. It flourishes in marshy districts, in deltas, on alluvial soils, and on the banks of tropical rivers—in fact, a proper degree of porosity, of temperature, and of humidity of soil favour the growth of this microbe : hence the reason that *B. malariæ* has been called 'an earth-born poison.'[1] This microbe is said to be heavier than most gases, 'and scarcely floats six feet above the ground; it may be wafted some distance by winds, but mountains hold it back, and belts of trees, especially the eucalyptus, destroy its efficacy.'

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[1] This eminent *savant* has been obliged to give up his important investigations. He wrote to the author as follows : 'I have been compelled to give up microscopical researches since 1886, because my eyes are almost ruined.'

[2] *Proceedings of Royal Society*, vol. xlvi. p. 542.

Gubler[2] and many others have shown that the eucalyptus or 'fever-destroying' tree has considerable power in destroying the microbe of malaria, this being due to the action of the aromatic gases given off by the tree. One instance may be cited of the fever-destroying properties of the eucalyptus. 'In a desolate part of the Campagna there stands an old monastic institution upon a spot consecrated by tradition as that whereon St. Paul was martyred. For centuries this part of the Campagna [Tre Fontane[3]] was a stronghold of pestilential fever, and prolonged residence in the monastic institution in question surely led to death. Some few years ago a band of Trappist monks planted the eucalyptus in its cloisters, and the trees have since grown to a great height. What is more important, however, is that the place is now once more habitable, and fever, it is said, reigns there no more.'[4] There are also plantations of the eucalyptus in Corsica, Algeria, Italy, California, Australia, and other parts of the world; and there is little doubt that these trees are antagonistic to the spread of malaria, because the

[1] Felkin in *Proc. Roy. Soc. of Edinburgh*, vol. xvi. p. 269.

[2] *Journal de Pharmacie et de Chimie*, 1871.

[3] Known anciently as Aquæ Salviæ.

[4] Kingzett's *Nature's Hygiene* (3rd ed.), page 266; see also Giglioli's *Fermenti e Microbi*, pp. 247-257.

essential oil secreted by the trees contains a hydrocarbon—$C_{10}H_{16}$; and as this is vapourised, it is resolved in the presence of atmospheric oxygen and moisture into camphoric peroxide, camphoric acid, and hydrogen dioxide[1]:—

$$(\alpha)\ 2\,C_{10}H_{16} + 5O_2 = 2C_{10}H_{14}O_4 + 2H_2O,$$
$$(\beta)\ C_{10}H_{14}O_4 + 2H_2O = C_{10}H_{16}O_4 + H_2O_2;$$

and it is the hydrogen dioxide, so produced, which destroys the microbia of malaria.

In the treatment of malaria certain medicinal substances are used. (1) Tommasi-Crudeli[2] recommends arsenious acid in small doses; and, according to many English authorities, Fowler's solution (containing 1 part of arsenious acid in 120 parts of water) should be prescribed in 5 to 10 minim doses three times a day. (2) Quinine salts, in large doses, have also been recommended, especially by travellers who have had to pass through malarial districts.

TYPHOID FEVER.

The microbe of this disease has been found in Peyer's glands, the spleen, larynx, lungs, liver, and in the lymphoid follicles of the intestine in fatal cases. Sometimes the microbe is present in the kidneys and urine. *Bacillus typhosus* measures from 2 to 3 μ long, and from 0·3 to 0·5 μ wide; and it forms filaments which sometimes measure 50 μ in length. It (Fig. 33, 4) has rounded ends; and it has been stated that spore-formation takes place at the extremities of the rods. This statement is, however, doubted by some bacteriologists; because the so-called spores have never been observed to germinate, etc. *B. typhosus* grows on bouillon, nutrient gelatine,[1] steamed potatoes (at 37°C.), and blood serum; and it can grow either in the presence or in the absence of free oxygen. On gelatine-plates, the microbe gives rise to greyish colonies with irregular margins, without liquefying the gelatine. In tube-cultivations, a growth appears as a bluish-grey film on the surface, whilst 'in the needle track there is a delicate zone of the same bluish-grey colour, surrounded in turn by a peculiar opalescent milkiness. The most characteristic growth, however, occurs on sterilised potatoes. It is characteristic in that, even when there is a most luxuriant growth of the typhoid bacillus, it cannot be recognised by the naked eye, even at the end of three or four days, except by a peculiar moist appearance of the potato, which, taken along with the appearances in milk and on gelatine, so far as is at present known, distinguishes the growth of this microbe from all others. It will be remembered, however, that the potato is slightly acid; and it appears that this acidity is necessary for this typical growth, for on potatoes rendered slightly alkaline there appears a yellowish or dirty grey growth with sharply-defined margins—a growth quite different from that above described.'

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[1] Mr. C. T. Kingzett, F.C.S., manufactures these substances on a large scale. He decomposes the essential oils (principally turpentine oil), in the presence of water, by passing a current of air into them, the products being sold as 'Sanitas' fluid and oil, both of which are powerful germicides. Kingzett imitates the decomposition of the essential oils by a similar process as the one which goes on naturally in the eucalyptus, pine, and camphor forests. It may be stated that 0·4 gramme of 'Sanitas' oil completely destroyed *Micrococcus prodigiosus*, *Bacterium allii*, *Bacillus tuberculosis*, and *Bacillus subtilis* when grown in various media as tube-cultivations.

[2] *Atti della R. Accademia dei Lincei*, 1885.

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[1] Gaffky in *Mitth. aus dem. k. Gesundheitsamte*, 1886.

Fraënkel and Simmonds[1] state that this microbe is the cause of typhoid fever, for they have produced the disease in monkeys, mice, and rabbits, by inoculation, from a pure cultivation of the microbe. Many other microbes (especially micrococci[2]) 'appear in the intestines when the disease is approaching its end, but *the bacillus* in question is the only one found in the blood and internal organs [as well as in the roseolous eruption], so that it is really characteristic of the disease.'

According to Janowski,[3] the action of light is detrimental to the growth of *B. typhosus*; and he has also proved that a temperature of 55°C., continued for ten minutes, destroyed the microbe. Although destroyed at 55° C., *B. typhosus* has been found alive in ice which had remained continuously frozen for a period of 103 days;[4] and Dornil has discovered that ice is often a medium for transmitting infectious diseases—especially typhoid fever. But if ice is a means of transmitting typhoid fever, potable water is a much more dangerous source of infection. 'The remarkable instance which occurred at the Caterham Waterworks (1879), where by the merest accident of one workman suffering from typhoid fever, who went down into the well and worked there a few hours, and defiled the well, thus contaminating hundreds of millions of gallons of water which were pumped out and distributed to the townspeople round about, four hundred cases of typhoid fever followed the next week, and seventy or eighty deaths occurred in consequence' (Hogg). Certainly this instance proves that water is a source of infection; but potable water is more frequently contaminated by the excreta of patients suffering from typhoid fever; and when such is the case, an epidemic of typhoid fever is the result of drinking such water. In 1874, an epidemic of this disease broke out at Over-Darwen, when 2035 persons were attacked, which terminated in 104 deaths. The outbreak was traced to the water supply. In 1884, a similar epidemic broke out at Zurich;[1] the origin of which was traced to the water of the river Limmat having been polluted with sewage containing typhoid-fever dejecta.

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[1] *Die Aetiologische Bedeutung des Typhus-bacillus*, 1886.

[2] Klein, *Reports of Medical Officer of the Privy Council*, 1875.

[3] *Centralblatt für Bakteriologie*, Bd. 8 (1890).

[4] F. Davis's *Handbook on Potable Water* (1891).

Epidemics of typhoid fever have also occurred at Florence,[2] Vienna, Rome, Naples, etc., which have been traced to potable waters having been contaminated with the evacuations of typhoid-fever patients.[3]

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[1] *Revue d'Hygiène*, 1885.

[2] Tommasi-Crudeli in *Istituto di Anat. Patologico* (Turin), 1882, p. 154.

[3] See also Thorne's *Reports to Medical Officer of Local Government Board*, 1880, *et seq.*; Cassedebat in *Comptes Rendus de l'Académie des Sciences*, vol. cx., and *Annales de l'Institut Pasteur*, 1890; Giglioli's *Fermenti e Microbi*, pp. 268-282; Dr. E. Frankland's *Experimental Researches in Pure, Applied, and*

As the stools or dejecta of typhoid-fever patients contain the typhoid bacilli, they are highly infectious; consequently they should always be disinfected before being thrown away. This would greatly interfere with the spread of the disease. Several authors have recommended carbolic acid or mercuric chloride for disinfecting the stools; but iron sulphate, according to Jalan de la Croix, is far more powerful than carbolic acid, and is only slightly inferior to mercuric chloride: besides, iron sulphate is a cheap disinfectant, non-poisonous and inodorous, and therefore may safely be recommended for the purpose of disinfecting the stools of patients suffering from typhoid fever and other infectious diseases. The author[1] has proved the high value of iron sulphate as a germicidal and fungicidal agent; and this compound readily destroys *Bacillus typhosus.* It may be stated that Dr. Proust[2] has used, for a number of years, iron sulphate to disinfect the stools in cases of typhoid fever.

Bacillus typhosus forms a ptomaïne, which has been extracted from pure cultures of the microbe, in glycerine-bouillon (3 : 100), by Brieger.[3] This

Physical Chemistry, p. 605; and S. T. Griffiths in the *Tamworth Herald,* August 15 and 22, 1891.

[1] *Proceedings of Royal Society of Edinburgh,* vol. xv.; *Journal of Chemical Society,* 1883-87; *Chemical News,* vols. xlvii.-lvi.; *Bulletin de la Société Chimique de Paris,* 1889, p. 667; *The Diseases of Crops* (G. Bell & Sons).

[2] *Traité d'Hygiène.*

[3] *Untersuchungen über Ptomaine,* 1886, p. 85; and *Virchow's Archiv,* 1889, p. 488. See also Gautier's *Chimie Biologique* (1892), p. 269.

base, which has been called typhotoxin ($C_7H_{17}NO_2$), dilates the pupil, produces diarrhœa, and rapidly kills animals. Luff[1] has also extracted a ptomaïne from the urine of typhoid fever patients; but no formula has been given to this base (*i.e.* it has not been submitted to quantitative analysis).

Dr. Lauder Brunton says, in regard to typhoid fever, that 'the symptoms do not point so much to the formation of a poison affecting the body generally, as to the local action of the microbes upon the intestines, although in some epidemics of typhoid fever the intestinal symptoms are but slightly marked, while bronchial irritation is due to the action of a microbe or to a ptomaïne produced by it on the bronchial mucous membrane.'

CHOLERA.

Since the great epidemic of 1832, cholera has had a peculiar fascination for those interested in the subject; for the disease has always been shrouded in mystery until recent times. 'Before the three last epidemics (1865, 1873, 1884) cholera usually came to Europe by what may be called the Continental routes—the caravan routes through Persia, Asia Minor, and Russia; but in the three last it came by the Mediterranean or maritime route, first by land through Egypt, brought there by Mecca pilgrims, and thence to the seaports of France, Italy, and Spain, whence it gradually made its way northward and inland, spreading over the whole of Europe.' The native habitat or the endemic area of this terrible disease is in India—especially in the delta of the Ganges. 'It can be readily understood, after the fearful ravages which it made in places in which it was not actually endemic, and after it had decimated the population in certain parts of India, in Egypt, in the low-lying portions of Persia, and Asia Minor, and in Europe, that many observers should be anxious to find out the ultimate cause of the disease; and as early as 1848 Virchow, and in 1849 Pouchet, Brittan, and Swaine found numbers of vibriones in the discharges of choleraic patients, without, however, being able to assign to them or prove for them any specific *rôle* in the causation of the disease.'[1] Since 1848, many scientists have been at work trying to establish a specific cause of cholera; but it was not until 1884 that Dr. R. Koch[2] discovered the comma bacillus in choleraic dejecta, etc. Although many distinguished pathologists have not accepted Koch's evidence of the bacillary nature of Asiatic cholera, there can be no doubt, after the important and extensive researches of Drs. Macleod and Milles,[3] that the comma bacillus of Koch is the cause (directly or indirectly) of Asiatic cholera.

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[1] *British Medical Journal*, 1889, p. 193.

The comma bacillus or *Spirillum choleræ Asiaticæ* measures from 1·5 to 2·5 μ long and 0·6 μ broad (Fig. 33, 3). It occurs singly, in pairs often S-shaped, in filaments which are screw-shaped, and in zoogl∞a, and it is motile and aërobic. Numbers of this microbe are found in the 'rice-water' stools formed by the desquamation of the mucous membrane of the intestines. They also occur in the intestinal follicles, and in the sub-epithelial spaces, and probably in the kidneys and urine.

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[1] Woodhead's *Bacteria and their Products*, p. 151 (W. Scott).

[2] *Deutsch. Med. Woch.*, 1884; *Berlin Klin. Woch.*, 1885.

[3] *Proceedings of Royal Society of Edinburgh*, vol. xvi. p. 18.

There are several other comma-shaped bacilli, but these differ in many respects from the microbe which Koch has so frequently found in choleraic dejecta. The following is the list of the other comma-shaped bacilli, with the names of their discoverers:—

(*a*) Finkler and Prior's bacillus (*Spirillum Finkleri*), found in cholera nostras. It is thicker than Koch's bacillus; and the colonies on gelatine plates are much larger than those of the comma bacillus of the same age. (*b*) Lewis's *Spirillum sputigenum* was found in the saliva; it is thicker than Koch's bacillus, and is quite distinct from the latter microbe. (*c*) Miller's bacillus was found in some cases of caries of the teeth; it is similar to Finkler's bacillus. (*d*) Kuisl's bacillus, found in human fæces, is also similar to Finkler's bacillus. (*e*) *Spirillum tyrogenum* (see Fig. 24) of Deneke is smaller than Koch's bacillus. It occurs in old cheeses, and, unlike the comma bacillus, it will not grow on steamed potatoes. (*f*) Klein's bacillus was found in some cases of diarrhœa, especially in monkeys. It grows differently in gelatine, giving rise to an offensive smell. (*g*) Ermengem and others have found comma-shaped bacilli in the intestines of guinea-pigs, pigs, rabbits, horses, etc., but, unlike Koch's bacillus, they will not grow in 10 per cent. gelatine. (*h*) Lingard found two kinds of comma-shaped bacilli in a case of noma, the smaller of which is said to have been similar to the choleraic one. (*i*) Gamaleïa's bacillus was found in a fatal fowl disease which was prevalent at Odessa. (*j*) Weibel found various forms in mucus, but their mode of growth is distinct.

Koch's *Spirillum choleræ Asiaticæ* is always present in Asiatic or malignant cholera, and it has not been found apart from this disease, and disappears from the body with the disease. Its habitat is the intestinal canal, and the detection of this bacillus enables the physician more readily to diagnose the earliest cases in an epidemic of cholera.

Ermengem,[1] Watson Cheyne,[2] Koch,[3] Nicati and Rietsch,[4] Macleod and Milles,[5] and others, have produced the disease in dogs and guinea-pigs by inoculation with pure sub-cultures of Koch's comma bacillus. The last two investigators have arrived at the following conclusions concerning cholera and its microbe:—

(*a*) The comma bacillus (Koch's) is always present and associated with certain changes in the small intestine in cases of Asiatic cholera. (*b*) There is no evidence to show that it is a *normal* inhabitant of the human alimentary canal, and therefore no proof of the assertion that it is a result of the disease. (*c*) The means used to introduce the comma bacillus into, and those used to lessen the peristalsis of, the small intestine of the guinea-pig, cannot be regarded as causing appearances like those of Asiatic cholera, or as causing the death of the animal, far less a mortality of over 60 per cent. (*d*) Pure cultivations of the microbe are pathogenic to the guinea-pig. (*e*) The contents of the ileum from those animals killed by injections of pure cultivations of the bacilli act in the same manner as pure cultivations of that microbe. (*f*) The microbe multiplies in the small intestine of the animal, and there is associated therewith changes similar to those in man in Asiatic cholera. (*g*) As there are conditions which favour the passage alive of the comma bacillus through the stomach of the guinea-pig, and also conditions which favour its multiplication in the small intestine of that animal; so in man, as there cannot be a doubt that the microbe finds conditions favourable to its multiplication in his small intestine, it must have found conditions favourable to its entrance alive through, in all probability, the mouth and the stomach (Macleod and Milles).

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[1] *Recherches sur le Microbe du Choléra Asiatique* (1885).

[2] *British Medical Journal*, 1885.

[3] 'Etiology of Cholera' in Laycock's *Microparasites and Disease.*

[4] *Revue d'Hygiène*, 1885 ; *Archives de Physiologie*, 1885.

[5] *Loc. cit.*, pp. 18-35.

The comma bacillus grows in neutral bouillon, gelatine, agar-agar, milk, and on steamed potatoes. It grows best if the medium is slightly alkaline, and at a temperature ranging from 16° to 40° C. On gelatine plates the colonies (Fig. 44) are evident in about twenty-four hours, and appear, under a low power, as small, somewhat irregular pale masses.

These gradually increase in size, and, where near the surface of the gelatine, a small depression forms over them, so that, on looking from the side at the surface of such a cultivation, it presents numerous little depressions instead of the original smooth surface of the gelatine, each depression corresponding to a colony of these bacilli. As the colony increases in size it becomes less compact, and the gelatine in the immediate vicinity becomes fluid.[1] At this stage zooglœa are formed. The colony goes on increasing in size for a few days, but ultimately ceases to extend, or extends only very slowly. Tube-cultivations are also characteristic. In twenty-four hours, at a temperature of 18° C., growth is evident along the needle-track as a whitish line, broader at the upper part, and gradually tapering to the lower. At the upper part of the gelatine there is a slight depression, and during the next twenty-four hours the growth becomes more marked, the depression increasing in size so as to look like

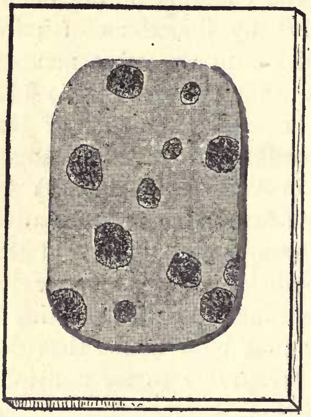

FIG. 44. COLONIES OF CHOLERA-BACILLI ON GELATINE-PLATE. (× 80.)

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[1] Dr. Lauder Brunton has shown that this liquefaction is due to a ferment (enzyme) secreted by the comma bacillus (*Proc. Roy. Soc.*, vol. xlvi. p. 542).

an air-bubble at the top of the track. In the following days the gelatine at the top becomes liquid, and this liquidity extends gradually to the bottom of the track, thus there is a funnel-shaped appearance from the greater amount of the fluid at the top than at the bottom. At the same time, the mass of bacilli falls to the bottom of the fluid and assumes a somewhat rosy colour, so that there is a rose-coloured convoluted string running down the lower part of the track. The fluid at the upper part, which in about a week has extended to the sides of the tube, becomes clear, except a very thin layer at the top, which remains opalescent, the top itself being often covered with a very fine scum. Scattered over the solid gelatine forming the sides of the funnel are seen numerous small irregular highly-refracting particles. These are the small zooglœa masses which have fallen to the sides and bottom of the funnel-shaped cavity (Fig. 45), and which Dr. Watson Cheyne considers the most typical appearance during the growth of the comma bacillus in tube cultivations. On agar-agar the comma bacillus grows fairly well, but it does not liquefy this medium. On blood serum (at 37° C.) this microbe grows most luxuriantly. It also grows in milk, but gives rise to no noticeable alterations;

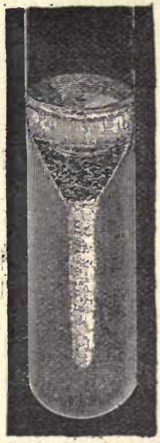

FIG. 45. TUBE-CULTIVATION OF CHOLERA-BACILLI. (*After Watson Cheyne.*)

'it may, therefore, be readily understood how deadly the cholera microbe may become if it once finds a resting-place in milk.'[1]

Brunton, Lewis, and Cunningham, Klebs and Cantani, and others, have all obtained indications of a poison or ptomaïne in cholera dejecta. Pouchet, Brieger, and Villiers have extracted several ptomaïnes from cholera dejecta, as well as from pure cultivations of the comma bacillus (Brieger).

Dr. Lauder Brunton[2] says, 'The symptoms occurring in cholera are probably due to the action on the tissues of a poison [or poisons] generated by the microbe, and not of the microbe itself, just as intoxication is due to the alcohol produced by the yeast plant, and not to the action of the plant itself on the nervous system and blood.' Besides the ptomaïnes produced by the comma bacillus, this microbe secretes a soluble enzyme.[3]

Cholera follows the course of rivers. Moisture in the atmosphere and the soil is needed for its distribution. Moist winds spread it, but the great factor in the distribution of cholera, as already stated, is human intercourse. Although human intercourse is the chief factor in distributing this disease, potable water is one of the most convenient vehicles for the distribution of the comma bacillus. If the dejecta of one or more choleraic patients contaminate a water supply, the water becomes a medium for spreading the disease. Such are the conclusions of Koch, Macnamara,[1] and many other observers. 'In India, in the regions in which cholera is endemic, the wells, as a rule, are merely surface tanks into which sewage and surface water may be drained, and which are frequently on the same level as, and connected with, the cesspools, so that even the water supply contains a considerable quantity of organic matter in which organisms of all kinds can flourish most luxuriantly; whilst these same wells, being merely dug-out pits beneath the slightly raised houses, are open for the reception of sewage and excreta of all kinds, especially in times of illness, when neither patients nor nurses have strength or time to see these are properly removed.' The recent epidemics of cholera in India, Spain, Japan,[2] and other countries, have been traced to the water supply;[3] and it is stated that the epidemic of 1884 killed 80,000 persons in Spain alone.[4] But it may be stated 'that with all the improvements that have been made in the drainage system and water supply of Lower Bengal, cholera has only diminished about 60 per cent., so that there still remain certain factors that favour the spread of cholera, and every now and again such a spread or outbreak may take place with extreme rapidity, and may involve a very wide area. Cleanliness, however, both general and personal, may be said to be the most important factor in *the prophylaxis* of cholera.'

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[1] Hence milk adulterated with water from districts in which there are persons suffering from cholora may be the means of causing an epidemic of the disease. The same may be said of typhoid fever.

[2] *Disorders of Digestion* (1888), p. 41; see also pp. 292 and 263; and *Practitioner*, 1884, *et seq.*

[3] See Dr. Brunton's paper in *Proc. Roy. Soc.*, vol. xlvi. p. 542; and Dr. G. E. C. Wood's paper in *Proc. Roy. Soc.*, *Edinburgh*, vol. xvii. p. 29.

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[1] *British Medical Journal*, 1884, p. 502.

[2] An epidemic of cholera or korera-byo (as the Japanese call it) occurred in Japan in 1890, and there were 13,141 deaths out of 21,116 cases (*vide* Sir Edwin Arnold's *Seas and Lands* [1891], p. 474).

[3] *Lancet*, 1885, *et seq.*

[4] Giglioli's *Fermenti e Microbi*, p. 300 *seq.*

It should be borne in mind that in cases of cholera, isolation and disinfection are absolutely necessary to prevent the disease spreading.[1] For further information on the subject of cholera and its microbe, the reader is referred to the undermentioned works.[2]

GLANDERS.

This contagious infective disease is caused by the *Bacillus mallei* (Fig. 33, 12), which has been found in the lungs, liver, spleen, and nasal membranes of horses and sheep dead or dying from glanders.

The same microbe has been found in human glanders or farcy; and the death of Dr. Hoffmann, of Vienna, in 1889 is a standing proof of the pathogenic nature of this microbe, and its being the cause of the disease known as glanders.[3] In man, this microbe has been found in the blood and pus of the ulcers. According to Löffler, glanders is essentially a disease of hot countries, 'where the comparatively high temperature appears to be extremely favourable to the development of the bacillus outside the body, especially in such materials as fodder, manure, and stable refuse generally. We have interesting evidence of this in statistics collected by Krabbe, who gives the following proportion of horses affected with glanders per annum per 100,000 horses in the following countries:—Norway, 6; Denmark, 8·5; Great Britain, 14; Sweden, 57; Wurtemburg, 77; Russia, 78; Servia, 95; Belgium, 138; the French Army, 1130; and the Algerian Army, 1548.'

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[1] Cameron's *The Cholera Microbe and How to meet it* (Baillière & Co.).

[2] Klein's *Bacteria in Asiatic Cholera*; Brunton's *Disorders of Digestion* (1888), p. 262; Thorne's 'Sea-Borne Cholera' in *British Medical Journal*, 1887; Straus, Roux, Nocard, and Thuillier in *Comptes Rendus de la Société de Biologie*, 1883; and Bellews's *History of Cholera in India* (1885).

Griffiths' *Researches on Micro-Organisms*, p. 15.

B. mallei measures from 2·5 to 5 μ long, and about one-fifth of its own length broad. It grows on blood serum (at 38° C.), sterilised potatoes (at 37° C.), in neutral solutions of extract of beef (at 37° C.), and in various vegetable infusions. Horses, asses, cats, rabbits, mice, and guinea-pigs, inoculated with a few drops of a pure cultivation of this microbe, have died with the characteristic lesions of glanders (glanderous ulcers and modules in the internal organs, and on the nasal septum).

Stables, in which glanders has occurred, should be thoroughly washed out with a 2-per cent. solution of carbolic acid or some other equally powerful disinfectant.

DIPHTHERIA.

Diphtheria is an extremely infectious disease which attacks man and certain animals.

Two microbes were originally isolated by Klebs and Löffler from human diphtheritic membranes; but Dr. Klein[1] has shown that the Klebs-Löffler bacillus No. 1 is not constant in diphtheritic membranes, does not act pathogenically on animals; and does not grow on solid gelatine at 20° C. In fact, this microbe has been termed the pseudo-diphtheria bacillus. The other species, Klebs-Löffler bacillus No. 2, is always present in diphtheritic membranes—in fact, it is present even in the deeper layers of the membranes in great masses, and almost in pure culture. This microbe acts virulently on animals, and grows on gelatine at 19-20° C. Klein considers this bacillus to be the true microbe of diphtheria (Fig. 46 A and B).

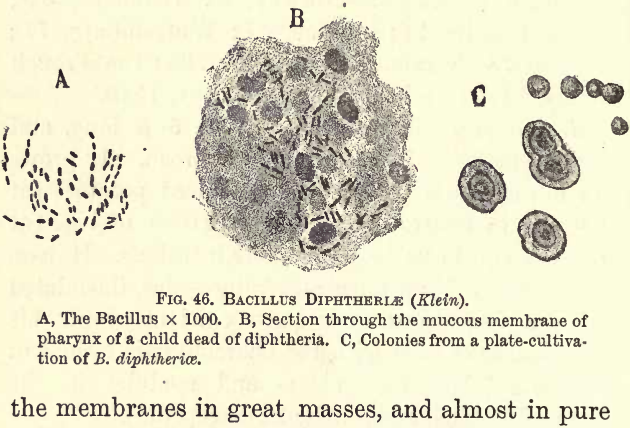

Fig. 46. Bacillus Diphtheriæ (*Klein*).
A, The Bacillus × 1000. B, Section through the mucous membrane of pharynx of a child dead of diphtheria. C, Colonies from a plate-cultivation of *B. diphtheriæ*.

On the slanting surface of gelatine in tubes,

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[1] 'Etiology of Diphtheria' in *Reports to Local Government Board*, 1889-90, p. 143; *Proc. Roy. Soc.*, 1890; *Centralblatt für Bakteriologie*, Bd. vii. (1890).

Bacillus diphtheriæ (No. 2) gives rise to greyish dots after 36-48 hours' incubation at 20° C. After three or four days, these appear as white round convex droplets, which ultimately aggregate together forming yellowish-brown colonies. Colonies are also formed when the microbe is grown as a plate-cultivation (Fig. 46 C). In alkaline bouillon, *B. diphtheriæ* gives rise to a turbidity in twenty-four hours after inoculation; and afterwards a greyish-white precipitate is produced at the bottom of the tube.

In milk kept at 18-20° C., this microbe grows very rapidly. The milk always remains fluid; but in two or three days after inoculation, flakes of casein separate.

B. diphtheriæ (from 3 to 6 μ long) does not produce spores; but it gives rise to a soluble enzyme and one or more ptomaïnes. Drs. Roux and Yersin[1] isolated an enzyme, from a pure cultivation of the microbe in question, which produces all the symptoms of diphtheria. This is a true enzyme, for boiling water destroys its action.

The author[2] extracted a ptomaïne ($C_{14}H_{17}N_2O_6$) from urine in cases of diphtheria; and the same ptomaïne was also obtained from pure cultures of *B. diphtheriæ* on peptonised gelatine. This ptomaïne is not present in normal urine. Brieger and Fraënkel[3] have also isolated a toxalbumin from pure cultivations of the microbe. This substance is said to have produced toxic effects when injected into animals. 'These observers, however, did not separate from the albumoses that were formed any enzymes that might be present, consequently they were working with a mixture of substances. The products that they obtained gave most of the reactions of albumoses; they were certainly toxic, but they probably contained both enzymes and albumoses' (Woodhead).

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[1] *Annales de l'Institut Pasteur*, 1888.

[2] Griffiths in *Comptes Rendus*, vol. cxiii. p. 656; *Nature*, vol. xlv. p. 72.

[3] *Berlin Klin. Woch.*, Bd. xxvii. pp. 241 and 1133.

B. diphtheriæ (No. 2), which is identical with those of Roux and Yersin, Zarniko, Escherich, and Löffler, acts very virulently on guinea-pigs on subcutaneous inoculation: at the seat of the injection a tumour is produced, which in its pathology and in microscopic sections, completely resembles the diphtheritic tissue of the human subject. In human diphtheria *B. diphtheriæ* is present only in the diphtheritic membrane, but neither in the blood nor in the diseased viscera; the same holds good of the experimental guinea-pigs. In subcutaneous inoculation with artificial culture, though it causes in these animals acute disease and death—the lungs, intestine, and kidney are greatly congested—the diphtheria bacillus remains limited to the seat of inoculation (Klein).

Klein has shown that this microbe also attacks the cat and cows, as well as man and the guinea-pig. But, unlike human diphtheria, the disease locates itself in the lungs of the cat (Fig. 47), *i.e.* the lung is the organ in which the diphtheritic process in the cat has its seat. The domestic cat is, therefore, a means of introducing diphtheria into a household.

Klein has also shown that a definite disease can be produced in the cow by the *B. diphtheriæ*, consisting of a diphtheritic tumour at the seat of inoculation with copious multiplication of the bacilli, a severe pneumonia, and necrotic change in the liver; the contagious nature of the vesicular eruption on the udder and excretion of the bacilli in the milk prove that in the cow the bacilli are absorbed as such into the system. The morphological characters and the pathogenic action of these bacilli from milk were exactly the same as those from human diphtheria. According to the same authority, 1 litre (1·76 pints) of milk contained between 30,000 and 40,000 bacilli; therefore, there is little doubt that cows suffering from diphtheria are capable of transmitting the disease to human beings by means of the milk; and human beings suffering from the same disease may also infect a milk-supply, and so spread the disease among the consumers of such milk.

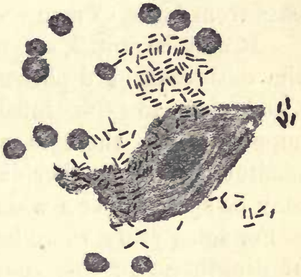

FIG. 47. BACILLUS DIPHTHERIÆ. (*Klein.*)

Represents a cover-glass preparation of fresh lung exudation from a cat that died of naturally acquired diphtheria in a house wherein diphtheria afterwards attacked the children of the household. (× 1000.)

Dr. G. Turner[1] states that fowls, turkeys, and pheasants also suffer from diphtheria, for he found the characteristic diphtheritic membranes in these birds; and he has also seen fowls and pigeons which had also been inoculated with diphtheritic membrane from a child's throat attacked with a disease which in all respects resembled what Turner regards as natural fowl-diphtheria. Similar accounts have been received from foreign bacteriologists,[1] so that the identity and transmissibility of the disease from fowls to men seems very probable.

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[1] *Reports to Local Government Board*, 1886-7, p. 3.

It may be stated, *en passant*, that Power[2] traced the outbreaks of diphtheria in 1886 at York and Camberley to the infectiousness of the milk-supplies; and there is no doubt that milk is a medium in which other diseases besides diphtheria may be spread over a wide area.

For some years, there has been a serious increase of diphtheria in this country, which Dr. Thorne[3] attributes to the increasing aggregation of children in elementary schools; and Dr. Seaton,[4] to the present systems of water-supply and sewerage.

B. diphtheriæ is possessed of great tenacity of life. If it is dried and kept at 33° C. it is still alive after three months; but at 45° C., this microbe is killed in four days. 'If a fragment of the false membrane containing bacilli be removed, wrapped in sterilised paper, or linen, and be carefully protected from the action of light, cultivations may be made from it at any time during a period of five months. If, however, instead of keeping it dry and in the dark, fragments of these membranes are exposed to the light and moistened and desiccated alternately, the virus is destroyed much more rapidly. From all this, and from the fact that the bacillus is destroyed by moist heat at 58° C., it is evident that by far the best method of disinfecting clothes, the floor, the walls, and furniture, is by the use of a liberal supply of boiling water; for although a temperature of 98° C. (dry), continued for an hour, is necessary to destroy the vitality of the bacillus, moist heat at a very much lower degree (acting only for a minute or two, according to the temperature), is sufficient to disinfect everything on which it is allowed to act' (Woodhead).

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[1] *British Medical Journal*, 1884; *Journal d'Hygiène*, 1884.

[2] *Report to Local Government Board*, 1886, p. 311.

[3] *Diphtheria: its Natural History and Prevention* (1891).

[4] *Report of International Congress of Hygiene*, 1891.

Drs. Behring and Kitasato[1] have recently discovered a method of producing immunity against diphtheria. As this is similar to Kitasato's method of treating tetanus, which has been already described (see p. 214), no further remarks are needed.

'Antiseptic throat washes,[2] not merely gargles, plenty of fresh air, and good nourishing food, are what are required in the treatment of diphtheria. Kill the germs as far as possible by means of the antiseptics [germicides], and strengthen the tissue cells by plenty of oxygen, and by promoting the excretion of effete products, by food and exercise, so that the cells shall be able to form their protective products, and shall also be able to play their part as phagocytes when called upon to do so.' It should be borne in mind that in diphtheria the bacilli are localised in the throat; but the poisonous products (ptomaïnes and enzyme), which the bacilli form, pass into the system. If the bacilli are destroyed by germicides,[1] these poisonous products cannot increase in the system; and if they have not already accumulated in too large a quantity, they are readily excreted. 'Another important point is that the disappearance of the bacilli from the mouth is not simultaneous with the removal of the false membrane, and Roux and Yersin have found that the specific bacillus may persist in the mouth for several days (in one case fourteen days) after all traces of the membrane have disappeared, and they give the good practical advice that diphtheritic patients who are becoming convalescent should not be allowed to associate with their school-fellows, play-mates, or families, for at least a fortnight after the membrane has disappeared; and that it is quite as important to wash out the throat freely three or four times a day with disinfecting lotions as that the clothes and bed linen should be thoroughly disinfected.'

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[1] *Deutsche Medicinische Wochenschrift*, 1890, p. 1113; and *Zeitschrift für Hygiene*, 1890-1.

[2] The following is an excellent antiseptic throat wash:—

℞. Potass. chlor. pulv., ʒ ij.
Acid hydroch. fort, ʒ j.
Let stand mixed for 10 minutes,
then add water gradually shaking
each time to . f. ℥ vi. }
Syrup, . . f. ℥ j. }

To be used with a spray apparatus or syringe. This fluid not only loosens the diphtheritic membrane, but also destroys the bacilli.

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[1] Dr. Wagner (*Jour. für Prakt. Chemie*, vol. xi.) has successfully used a solution of salicylic acid in the treatment of diphtheria.

TUBERCULOSIS.

Tuberculosis, in its varied protean guises, is one of the most widespread and deadly diseases in these northern latitudes. It has been stated that at any given time there are 200,000 persons in this country suffering from *phthisis pulmonalis*—the commonest form of the disease—and in each year nearly 70,000 persons die from it. The following tables show the death-rates per million from tuberculosis at different ages:—

(*a*) *From Phthisis.*

	Age 10.	Age 15.	Age 20.	Age 25.	Age 35.	Age 65.	Age 75.
Males, . . .	628	2093	3687	3941	4089	2152	752
Females, . .	1077	3019	3809	4175	3842	1364	546

(*b*) *From other Tubercular Diseases.*

	Age 5.	Age 10.	Age 35.	Age 75.
Males, ...	5008	641	103	94
Females, . . .	3942	515	98	89

Tuberculosis is known by various names, according to the parts of the body the disease may happen to attack, or according to the kind of lesions it produces, or, finally, according to its general effect on the body. Thus phthisis or consumption, lupus, caseous pneumonia, cheesy inflammation of the lungs, consumption of the intestines, tabes mesenterica, tubercular pleurisy, ceseous broncho-pneumonia, scrofula, tubercular meningitis, etc., are all forms of the same disease, which is produced by a microbe—*Bacillus tuberculosis*—discovered by Professor R. Koch[1] in 1882. This bacillus lives in the blood and tissues, and gives rise to tubercles, which are small abnormal nodules of newly-formed tissue studding the diseased organ or organs. Each tubercle is made up of nucleated cells and tubercle bacilli, the latter being located chiefly in the giant cells. As the tubercles are continually being thrown off from the diseased person or animal, tuberculosis is an infectious disease. *B. tuberculosis* attacks other animals besides man; among these may be mentioned cows, fowls, rodents, pigs, etc. Although tuberculosis is essentially the result of the action of Koch's bacillus, there are certain factors which render man and animals liable to contract the disease, and thereby receive the poison. These factors are deficiency of oxygen by bad ventilation, foods (from tuberculous animals), certain diseases,[2] starvation, inheritance, predisposition, etc. The last-named factor may be acquired through the system being of a lower standard than usual, or may be inherited.

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[1] *Berliner Klin. Wochenschrift*, Bd. xv. p. 221.

[2] Among the diseases which render man liable to contract tuberculosis are syphilis, diabetes, measles, whooping-cough, etc.

Tuberculosis, or that form of the disease known as phthisis (consumption), runs through certain families. There are two theories which account for the inheritance of phthisis—(*a*) that the tissues of children born of phthisical parents are especially favourable to nourish the tubercle bacilli; *i.e.* the tissues form a fertile soil for the subsequent growth of the microbes; (*b*) that the tubercle bacilli are actually contained in the ovum or among the spermatozoa, and so become a constituent part of the embryo and fœtus which develops within the uterus. Baumgarten records the fact that he has observed the tubercle bacilli in the ovum of the rabbit, and many observers have frequently seen the bacilli mingled with active spermatozoa. Professor Johne, of Dresden, discovered numerous tubercles in the lungs of a fœtal calf of seven months intra-uterine growth. This proves that if the ovum had not been inoculated, it received the virus (*i.e.* the tubercle bacilli) through the placenta, which amounts practically to the same thing. Similar intra-uterine inoculation has been shown to be more than probable in the human being; and Professor Burdon Sanderson[1] believes that many cases of phthisis are congenital, *i.e.* dependent on causes which have operated before birth.

Besides being hereditary, tuberculosis is also infectious, *i.e.* the disease is capable of being transmitted by direct or indirect infection from one host to another.

There are four modes in which the tubercle bacilli enter the body, viz., by pulmonary inhalation (atmospheric infection), swallowing (enteric infection), direct inoculation, and heredity. (*a*) *Inhalaton* is the commonest mode of infection. Koch and numerous other observers have proved that animals, after a few inhalations of phthisical sputum, disseminated in a spray, readily become infected with tuberculosis. Ransome[1] has isolated the tubercle bacilli from the breath of patients suffering from advanced phthisis; and the author[2] has confirmed Ransome's investigations; therefore it will be seen that tuberculosis may pass from husband to wife, and *vice versâ*; and it may also affect members of the same family, not because of any hereditary taint, but through the simple fact of close companionship.[3] The sputa or expectorations of phthisical patients are highly infectious, even after being desiccated for several months. *Bacillus tuberculosis* is often to be found in places lived in by consumptives; and Prausnitz has lately collected the dust in various compartments of trains which often convey patients from Berlin to Meran, and inoculated a number of guinea-pigs with it. Two, out of five compartments so examined, were found to contain the bacillus; the dust of one rendered three out of four guinea-pigs tuberculous, while that of the other compartment infected two of these animals. The animals were killed after several months, and their organs had developed tubercles containing the characteristic bacilli. (*b*) *Swallowing*, or enteric infection, is a means of introducing the tubercular virus into the animal economy. Rabbits, guinea-pigs, fowls, pigs, etc., become tubercular when fed upon tubercular tissues, sputum, saliva, milk, pure cultivations of the tubercle bacilli, etc. Klebs, Arloing, Chauveau, Villemin, Gerlach, Baumgarten, and others have shown, by direct experiment, that the milk, flesh, etc., 'from cattle affected with tuberculosis would, when introduced alone or along with other food into the alimentary canal of rabbits, etc., give rise to tuberculosis in the pharynx, in the lymphatic glands of the neck, the stomach, intestine, omentum, liver, and spleen, and then, later, in other organs.' Many authorities state that the flesh of tuberculous animals (cattle, fowls, pigs, etc.) give rise to tuberculosis in human beings. On the other hand, there are authorities which state that there is not much danger of human beings contracting tuberculosis from eating meat from tuberculous cattle; but it is a unanimous opinion among all competent authorities that the *milk* of tuberculous cows is a source of great danger to human beings—often giving rise to tuberculosis, especially in children. It should be borne in mind that 'boiling always destroys the virulence, even when the milk contains bacilli, which is the case when the udder of the affected cow is itself tuberculous;' and the risk of infection is greatly diminished, if not abolished, when meat from tuberculous cattle is thoroughly cooked.

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[1] *Report of International Congress of Hygiene*, 1891.

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[1] *Proc. Roy. Soc.*, 1882.

[2] *Proc. Roy. Soc. Edinburgh*, vol. xvii. p. 268.

[3] See Weber's book, *The Communicability of Consumption from Husband to Wife;* and Heron's *Evidences of the Communicability of Consumption.*

The experiments of Galtier, Bang, and others have proved that the various products derived from milk —butter, cheese, and butter-milk—may all contain the tubercle bacilli, and that these retain their vitality in such products for a period of from fourteen to thirty days. The majority of these bacilli may be separated from milk if the cream is first removed by means of a centrifugal machine, but if the milk is very rich in bacilli a few usually remain in the milk, and even in the cream. In order to do away with this danger, it is necessary to expose the milk or the cream before churning to a temperature high enough to kill the tubercle bacilli (85° C. for about five minutes). (*c*) *Direct inoculation* is the third mode of infection. When tubercular matter or pure cultivations of the tubercle bacilli are introduced beneath the skin of susceptible animals, such as rabbits, guinea-pigs, cats, etc., they always produce, in four or more weeks, the typical tubercular lesions — swollen lymphatic glands, tubercles in the spleen, liver, and lungs, and enlargement and caseation of the bronchial glands. Besides, there are instances recorded in which sores on the udder of cows have infected with tuberculosis the hands of the persons milking them; and it is not improbable that the common house-fly may disseminate the virus of phthisis by inoculating open sores on the hands and face (Spillman and Haushalter[1]).

Bacillus tuberculosis measures from 2 to 8 μ long and about 0·2 μ broad. It occurs in phthisical sputum (Fig. 48), in the cells of tubercles, and in the blood,[1] tissues, urine,[2] fæces, saliva,[3] and sweat[4] of tuberculous patients. Watson Cheyne[5] and other observers believe that the microbe is a spore-producing bacillus; but this assertion is doubted by Lankester[6] and others. *B. tuberculosis* has been cultivated artificially, and it has been proved that the strength of its virulence is not lessened by successive cultivations. When inoculated into various animals it always produces tuberculosis. The presence of this microbe in the sputa of patients supposed to be suffering from phthisis is a certain diagnosis; and it may be mentioned that the microbes are most numerous in the small caseous dots contained in the sputa. These dots should be searched for, then crushed between two cover glasses, dried, stained, and examined with high powers.

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[1] *Comptes Rendus*, vol. cv.

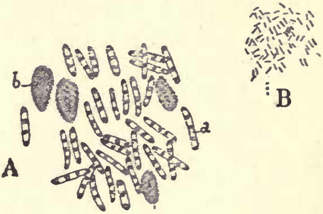

FIG. 48. BACILLUS TUBERCULOSIS.
A, From human sputum. a, Bacilli. b, Nuclei, × 1500. B, Bacilli, × 435.

B. tuberculosis grows on solid blood serum at 37° C. (*i.e.* the temperature of the body), and in eight or ten days after inoculation gives rise to whitish or yellowish drops or 'scales.' There is no liquefaction of the medium if the culture is perfectly pure. The bacillus also grows on the surface of bouillon (containing glycerine), forming a delicate thin film. Pawlowsky[1] has grown the tubercle bacillus on sterilised potatoes; but to succeed with this medium a considerable quantity of moisture must be kept in contact with the growing microbe. Nocard and Roux[2] have shown that most luxuriant growths of the tubercle bacillus are readily obtained when the microbe is grown on agar-agar and blood serum to which 6-8 per cent. of glycerine has been added; but after many successive cultivations on these glycerine media, the virulence of the microbe becomes distinctly diminished.

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[1] Weichselbaum in *Wiener Med. Blätter*, 1884.

[2] Babès in *Centralblatt für d. Med. Wissensch.*, 1883, p. 145.

[3] Griffiths in *Proc. Roy. Soc., Edinburgh*, vol. xv. p. 44.

[4] Griffiths' *Researches on Micro-Organisms*, p. 268.

[5] *The Practitioner*, 1883, p. 248.

[6] *Nature*, 1884.

B. tuberculosis forms cellulose in the organs and blood of tuberculous persons;[3] and it has been recently stated that the microbe, when growing in glycerine bouillon, produces an albumose.[4] The tubercle bacillus has great tenacity of life, for the author[5] has shown that it is capable of being dried up for three or four months at a temperature of 32° C. without losing its vitality: and Cornil was able to demonstrate that at the ordinary temperature of the room the tubercle bacillus, kept in water from the Seine, still retained its vitality after seventy

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[1] *Annales de l'Institut Pasteur*, 1888-9.

[2] *Annales de l'Institut Pasteur*, 1887, p. 19.

[3] See the author's *Researches on Micro-Organisms*, p. 155.

[4] Crookshank and Herroun in *British Medical Journal*, 1891, p. 401.

[5] *Proc. Roy. Soc. Edinburgh*, vol. xv. p. 42.

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days' immersion in that medium. As already stated the best temperature for the growth of this bacillus is 37° C.; at 40° C. its activity is diminished; and at a temperature ranging from 50° to 60° C. it is killed. Boiling or strongly heating cultivations of all microbes destroys them, or, in other words, the media so treated become sterilised. Goethe knew nothing about microbes, yet, with the genius of a great poet, he makes Mephisto say:—

> 'Der Luft, dem Wasser, wie der Erden
> Entwinden tausend Keime sich,
> Im Trocknen, Feuchten, Warmen, Kalten!
> Hätt' ich mir nicht die Flamme vorbehalten,
> Ich hätte nichts Aparts für mich.'

In addition to the action of heat, sulphuretted hydrogen, ozone, a solution of salicylic acid, and the electric current (E.M.F. of 2·16 volts), all destroy the vitality of *Bacillus tuberculosis*.[1]

Although it is out of place to discuss the methods used in the treatment of infectious diseases in a manual devoted to general bacteriology, we give a very brief account of what is known as 'Koch's cure' for tuberculosis. Ever since Dr. Koch discovered the tubercle bacillus (in 1882) he has been endeavouring to obtain an inoculating fluid which would kill the bacilli, and bring about a sufficiently strong and healthy reaction to expel them from the body without, at the same time, destroying healthy organs. Such a fluid Koch believes he has discovered in his tuberculin,[2] which is a glycerine extract from pure cultivations of destroyed tubercle bacilli. This so-called lymph contains mineral salts, colouring substances, unknown extractive matter, besides the dead bacilli. According to Koch, some of these substances can be removed from the 'lymph' tolerably easily. The effective substance is mainly insoluble in absolute alcohol, and can be precipitated by it, not, indeed, in a pure condition, but still combined with the other extractive matter, which is also soluble in alcohol. The colouring matter may also be removed, so that it is possible to obtain from the extract a colourless dry substance, which contains the effective substance in a much more concentrated form than the original glycerine solution. The effective substance appears to be a derivative from albuminous compounds, and is closely allied to them. It is not a ptomaïne; but appears to be an enzyme; and tuberculin contains less than 1 per cent. of this enzyme.[1]

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[1] See the author's book, *loc. cit.*, pp. 176, 182, 184, and 227.

[2] *Deutsche Medizinische Wochenschrift*, Nov. 14, 1890, and Jan. 15, 1891.

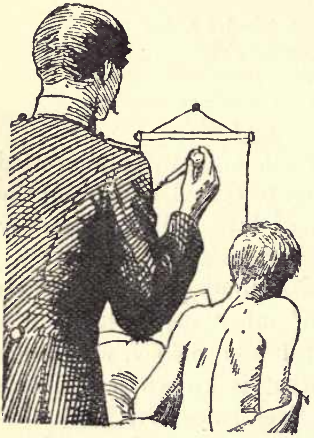

FIG. 49. INJECTING KOCH'S 'LYMPH.'

The treatment consists in injecting, subcutaneously, small doses[1] of diluted (with water) tuberculin into the back of patients (Fig. 49) suffering from certain forms of tuberculosis; and as the treatment progresses the doses are slowly increased 'as long as there may be bacilli in the body.' Koch's 'lymph' does not kill the tubercle bacilli, but destroys the tuberculous tissues, and thereby starves the bacilli contained in such tissues. It also sets up a localised reaction in the vicinity of the bacilli, by means of which the cells are so strengthened that they are able to prevent the extension of the bacilli into the surrounding parts; in fact there is a battle between the cells and the bacilli, and if the former are strengthened, it is possible for them to destroy the latter; and this is what Koch's 'lymph' is believed to do.

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[1] See also Hunter's paper in *British Medical Journal*, 1891 (ii), p. 169.

As to the value of Koch's treatment, there is no decided opinion among those best capable of judging; for some authorities are against, while others are in favour of, the 'lymph' as a diagnostic and curative agent. Professor R. Virchow[2] (the greatest living pathologist) 'has found, in a number of cases that have come under his observations,'—a comparative small number when the enormous number that have been injected is taken into consideration,—'that the characteristic degeneration of the tissues of the young tubercle is not always brought about, that the localisation of the disease is not by any means perfect, that there is a tendency of tuberculous material that should be thrown off to continue the infection and even increase its rapidity of spreading, especially in the lungs, and that in some cases the bacilli, instead of being rendered inert, appear to take on greater activity, and to be carried in the various currents in the body, even to parts situated at some distance from the original tuberculous focus.' According to Dr. Cornil, tuberculous affections of the skin are ameliorated by Koch's remedy, but it should be sparingly employed in the incipient stages of phthisis; and it is useless, and even dangerous, in advanced and acute cases of phthisis. Nevertheless, Professor Koch has made a great advance in the therapeutic treatment of infectious diseases.[1]

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[1] 0·0005 to 0·01 cc.

[2] *Berliner Klinische Wochenschrift*, Jan. 21, 1891, p. 49.

ANTHRAX.

The disease known as anthrax, splenic fever, splenic apoplexy, or malignant pustule, is a disease affecting man and animals. 'In some countries the losses to agriculturists and farmers owing to the fatal character of the disease in sheep and cattle is enormous. In man it is chiefly known among woolsorters and those engaged in the handling of hides. This disease has been definitely proved to be due to the *Bacillus anthracis,* which, after its entry into the system of an animal or human being, multiplies very rapidly in the blood and spleen, and, as a rule, produces a fatal result, at any rate in sheep and cattle.'

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[1] Various methods for treating phthisis are detailed in the author's book: *Researches on Micro-Organisms,* pp. 286-319 (Baillière & Co.); and see also Dr. Drewitt's paper in *Trans. Clin. Soc.,* 1887. Drewitt treated a child suffering from lupus partly by scraping and partly by salicylic acid.

Bacillus anthracis measures from 5 to 20 μ long, and from 1 to 1·25 μ broad (Fig. 50), and often occurs in masses of filamentous threads. It produces oval spores, and when either the bacillus or its spores are injected into mice, guinea-pigs, sheep, rabbits, etc., they die with all the characteristic lesions, etc., of anthrax. Even the inhalation of the spores is capable of giving rise to anthrax in man and susceptible animals. *B. anthracis* has been found in the blood, spleen, and other organs, also in the urine and fæces of animals suffering from or

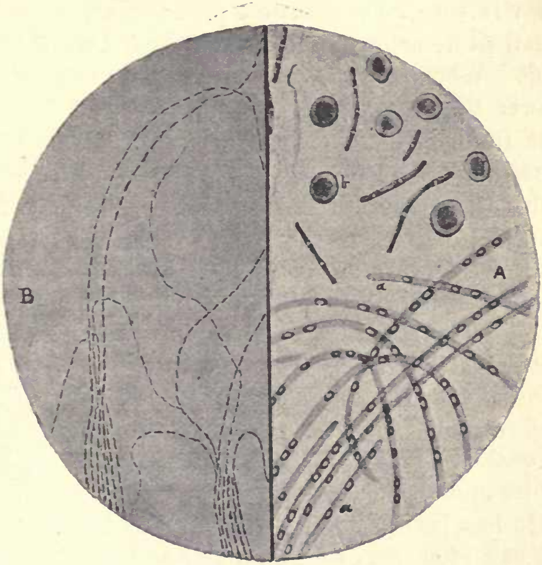

FIG. 50. BACILLUS ANTHRACIS.
A, Bacilli (*a*) forming spores (× 1200).
B, Convolutions of bacillary threads (× 320).

dead of anthrax. This microbe grows in nutrient gelatine, agar-agar, neutral bouillon, and on steamed potatoes at all temperatures between 15° and 43° C., best between 25° and 40° C. Free access of air is essential for *B. anthracis* to produce spores. Successive cultivations of this microbe do not weaken its virulence. On gelatine plates it gives rise to small white colonies after two or three days' incubation. When these colonies are examined under low power they appear as masses of twisted threads, but in cover-glass preparations (Fig. 50 B) these thread-like filaments are readily observed. In tube-cultivations the bacillus presents a characteristic appearance. Along the track of the needle there appear lateral growths which give the culture a peculiar feather-like appearance. But after a time the gelatine liquefies, and the growth sinks to the bottom of the tube, where the bacilli undergo degeneration. On agar-agar a similar appearance is presented, but there is no liquefaction of the medium. *B. anthracis* grows on steamed potatoes as a creamy-white granular mass.

It has been stated that the anthrax bacillus produces a ptomaïne called anthracin and an albumose[1] from the medium on which it lives.

Klein and Parsons[2] have shown that anthrax bacilli without spores are destroyed in five minutes when exposed to a temperature of 103° C. (dry heat), but the spores are not destroyed until they have been exposed to a temperature of 104° C. for four hours (dry heat). However, boiling in water for only one minute was sufficient to render inert the spores of *B. anthracis.*

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[1] Hankin in *Proc. Roy. Soc.*, 1890, p. 93; and Martin in *Nature*, vol. xlii. p. 118.

[2] *Report to Medical Officer of Local Government Board*, 1884.

According to MM. Chamberllent and Moussons,[1] anthrax bacilli have been discovered in the milk of cows affected with the disease, and not only is milk a means of giving rise to an outbreak of anthrax, but polluted drinking water derived from wells may also spread the disease.

As already stated, successive cultivations do not weaken the virulence of *Bacillus anthracis,* but if the microbe is cultivated in neutral bouillon at 42° or 43° C. for twenty days an attenuated virus is obtained. Pasteur's *premier vaccin* protects animals against the disease; but to make them perfectly refractory, they are inoculated a second time with a vaccine (*deuxième vaccin*) of less strength. Attenuated viruses for the protective inoculation against anthrax have also been obtained by exposing the bacilli to a temperature of 55° C., or to an aqueous solution of carbolic acid (0·5 to 1 per cent.), or sulphuric acid in a diluted form, as well as other chemicals. According to Hankin,[2] immunity against anthrax is obtained by inoculation with the albumose derived from pure cultivations of the bacilli, and he has also cured animals suffering from anthrax by injecting the albumose into their bodies.

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[1] *Comptes Rendus,* vol. cvii. p. 142.

[2] *Report of British Association,* 1890; and *British Medical Journal,* 1890.

ACTINOMYCOSIS.

This disease attacks cattle and occasionally man himself. It is caused by the ray-fungus or *Actinomyces.* ‘In cattle the disease manifests itself by firm tumours in the jaw, in the alveoli of the teeth, and particularly by a great enlargement and induration of the tongue—‘*wooden tongue.*’ Occasionally these tumours occur in the skin and lungs. The ray-fungus has been cultivated on solid ox-serum, and when pure cultures are injected into animals they give rise to actinomycosis.

THRUSH.

This disease is caused by the fungus *Oïdium albicans.* It is found on the mucous membrane of the mouth of infants, causing white patches on the tongue, gums, and soft palate. Like the higher fungi, this plant is composed of hyphæ and spores, which take root in the mucous lining of the mouth. The spores are produced by the division of the terminal cells, or sometimes by endogenous formation within the hyphæ.

In concluding the present chapter we may say that most infectious diseases have a microbian origin, but there are some (*e.g.* typhus fever, whooping-cough, mumps, etc.) in which no microbes have been isolated and cultivated apart from the body; and there are other infectious diseases which owe their origin to small *animal* organisms, known as

Protozoa. Dysentery and tropical abscess of the liver are due to *Amœbœ,*[1] and in India a fatal disease (surra), which attacks horses, mules, and camels, is caused by one of the *Flagellata.*[1]

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[1] See Dr. A. B. Griffiths' book, *The Physiology of the Invertebrata* (Reeve and Co.).

CHAPTER VII

THE MICROBES OF THE AIR

'THE solid matter floating in the atmosphere is every day becoming of greater and greater interest as we are gradually realising the important part it plays in the economy of nature, whether viewed as to its physical, physiological, or meteorological aspects. One fundamental point on which we have at present very little information of anything like a definite character is as to the number of solid particles present in the atmosphere. We know that they are very numerous, and it seems probable that the number varies under different conditions of weather, but what number of particles are really present under any conditions, and how the number varies, we have at present very little idea. In this field of research the physiologists are far in advance of the physicists, as they have devised means of counting the number of live germs floating in the atmosphere, and already we have a good deal of information as to how the number varies under different conditions.'

Before describing the living particles in the atmosphere we allude to some recent investigations on the number of dead or inorganic particles contained in the air. Mr. J. Aitken, F.R.S.,[1] has invented an ingenious apparatus by which the number of dust particles in the atmosphere may be readily estimated. Among the results obtained are the following:—

No. of Dust Particles in Air.

Source of air.	No. per cc.	No. per cubic in.
Outside (raining) .	32,000	521,000
Outside (fair) . .	130,000	1,119,000
Room . . .	1,860,000	30,318,000
Room near ceiling .	5,420,190	88,346,000
Bunsen flame . .	30,000,000	489,000,000

These results indicate that 'there is most dust in the air during dry weather, and perhaps during anti-cyclonic conditions, and least during wet weather, and perhaps in cyclonic areas.'

Aitken has also ascertained the minimum and maximum number of dust particles per cubic centimetre (cc.) in the air of various towns, etc. Among these results are the following:—

At Hyères (near Toulon), .	from	5000	to	46,000
,, Cannes, . .	,,	1550	,,	150,000
,, Lucerne (mountain air),	,,	210	,,	2350
,, Paris, . . .	,,	92,000	,,	210,000
,, London, . .	,,	48,000	,,	150,000
,, Ben Nevis (mountain air),	,,	335	,,	473
,, Dumfries, . .	,,	395	,,	11,500
,, Mentone, . .	,,	1200	,,	7200

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[1] *Transactions of Royal Society of Edinburgh*, vol. xxxv. p. 1; *Proceedings of Royal Society of Edinburgh*, vol. xvii. p. 193, and vol. xviii. pp. 39 and 259.

Aitken concludes (1) that the earth's atmosphere is greatly polluted with dust produced by human agency; (2) that this dust is carried to considerable elevations by the hot air rising over cities, by the hot and moist air rising from sun-heated areas of the earth's surface, and by winds driving the dusty air up the slopes of hills; (3) that none of the tests made of the Mediterranean *sea air* show it to be very free from dust; and (4) that the amount of dust in the atmosphere of pure country districts varies with the velocity and the direction of the wind: fall of wind being accompanied by an increase in dust. Winds blowing from populous districts generally bring dusty air.

It is stated that a man in the town inhales about 37,500 germs every twenty-four hours, and no fewer than 2,250,000 inorganic particles every minute.[1] 'Most of these are merely annoying, though a few are real messengers of disease and death. If the lungs are warm and moist, they can repel the particles; but with cold and dry lungs the suffering from the clogging must soon begin.'

Besides the inorganic or dead particles, the air is more or less laden with living particles. The majority of these are of the non-pathogenic or harmless kind, but there is plenty of evidence to show that pathogenic microbes lurk about in the atmosphere, and that many infectious diseases are propagated by means of air-carried microbes. Hence the reason that the study of aërial microbes is peculiarly interesting and attractive. The investigations of Burdon Sanderson, Tyndall, Lister, and Lankester have all thrown considerable light upon the conditions of life of these lower organisms; but Pasteur was the first investigator who made a systematic study of the presence and distribution of microbes in the atmosphere.

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[1] A cigarette smoker sends no fewer than 4,000,000,000 of particles (more or less) into the air with every puff he makes.

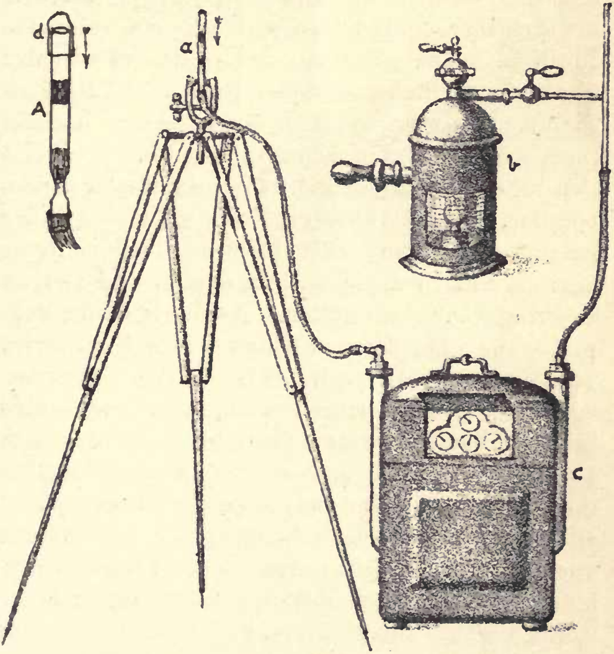

Fig. 51. Miquel and De Freudenreich's Filter.
A, Filter-tube (*A*, enlargement of same, with cap at *d*).
B, Aspirator. C, Gasometer.

It was not, however, until 1879 that Drs. Miquel and De Freudenreich attempted the quantitative estimation of aërial microbes. Their method consists in aspirating a known volume of air through a tube containing previously sterilised plugs of glass-wool (Fig. 51). The solid particles, including any microbes, are arrested; and the plugs of glass-wool are then thoroughly mixed with a known volume of sterilised water. The mixture is now sub-divided into such a number of equal parts that each part shall contain not more than one microbe. Each of these sub-divisions is then introduced into a cultivation tube or flask (see Fig. 17) containing sterilised bouillon. These tubes or flasks are placed in an incubator, and any that have received a living microbe will, in a short time, exhibit the fact by suffering visible alteration. As an example, supposing the plug through which twenty litres of air were drawn, by the aspirator (Fig. 51 *b*), was mixed with 25 cc. of sterilised water, and twenty-five tubes of bouillon were then each inoculated with 1 cc. of this mixture, and if, after a suitable incubation, it was found that only sixteen of them suffered alteration, it would be concluded that only sixteen microbes were present in the 25 cc. of water distributed among the twenty-five tubes, or, in other words, that the twenty litres of air contained sixteen living microbes.

Miquel and De Freudenreich have since substituted soluble media (powdered sugar or de-hydrated sodium sulphate) for the insoluble glass-wool. By the use of soluble filtering media, there is no chance of any microbes becoming imprisoned, as is the case when glass-wool is used. Drs. Miquel,[1] Fol,[2] Gautier,[3] and other French bacteriologists use soluble filtering media; and bouillon as the medium for the growth of microbes.

In England and Germany solid cultivation media have been substituted for the liquid bouillon; and when the microbian mixture is introduced into melted nutrient gelatine, it 'can be evenly dispersed throughout the medium by gentle agitation, and by subsequently allowing it to solidify, the microbes are not only isolated, but rigidly confined to one spot. Thus each individual microbe becomes a centre round which extensive multiplication takes place, and in a few days definite points of growth are visible to the naked eye, which are appropriately described as *colonies.* Although each colony consists of many thousands, or even millions of individual microbes, yet, as in the first instance, they owe their origin to a single organism or indivisible group of organisms, it is correct to regard the number of colonies as representing the number of microbes.'

One of the best methods for estimating the number of microbes in a known volume of air, is that devised by Dr. W. Hesse.[4] Hesse's method consists in slowly aspirating a known volume of air through a glass tube (28 × 1⅜ in.) which has previously been coated internally with a film of sterilised nutrient gelatine. The microbes suspended in the air are rapidly deposited within the tube, and on the surface of the gelatine give rise to colonies. Fig. 52 represents Hesse's aëroscope. At D is an indiarubber stopper, perforated to admit a small glass

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[1] *Annuaire de l'Observatoire de Montsouris*, 1880-92.

[2] *La Nature*, 1885.

[3] *Revue Scientifique*, 1886.

[4] *Mittheilungen aus dem kaiserlichen Gesundheitsamte*, vol. ii.

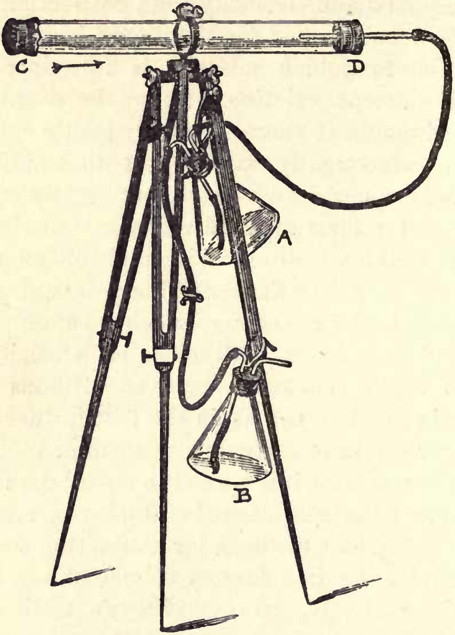

Fig. 52. Hesse's Aeroscope.

tube, plugged with cotton-wool; and at the opposite end is a perforated indiarubber cap, which is covered by an imperforated cap (C) of the same material. The aspirator consists of two like flasks (A B); one of which is filled with water. These flasks are reversible; but the one containing the water is always fixed uppermost when the air is passing through the tube. The down-flow of water causes the air to pass slowly through the tube when the outer cap (C) has been removed; and as the flasks are of known capacity, two, five, ten, or more litres of air may be aspirated through the tube. After this the cap is replaced, and the tube is then removed to a warm situation for several days, in order that colonies may develop.

Before introducing the nutrient gelatine, the tube, caps, and plug are sterilised by means of a solution of mercuric chloride, and finally with alcohol. After this treatment, 50 cc. of melted nutrient gelatine are poured into the tube, which is then sterilised in a steamer by the discontinuous method.

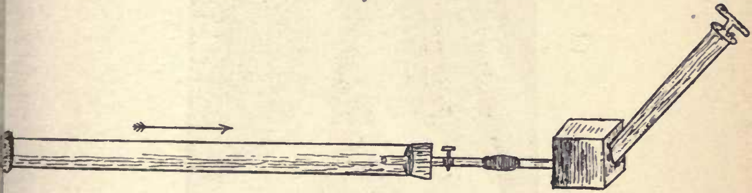

FIG. 53. GRIFFITHS' MODIFICATION OF HESSE'S AEROSCOPE.

The author has made a modification of Hesse's apparatus (Fig. 53), by substituting a small exhaust pump of known capacity for the aspirator. This modification is far handier and occupies less space than Hesse's aëroscope; while it gives results which agree with those obtained with the original apparatus. The late Dr. T. Carnelley[1] also modified Hesse's aëroscope by substituting a flat-bottom flask for the tube.

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[1] *Report of British Association*, 1887, p. 654.

Dr. P. F. Frankland[1] has devised a method by which a known volume of air is drawn, by means of an air-pump, through a short glass tube (4 × ¼ in.)

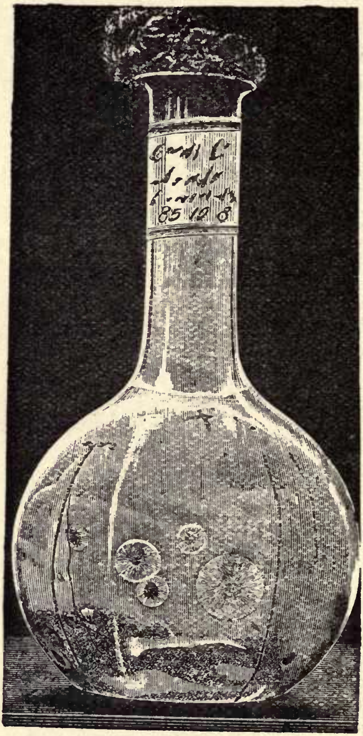

Fig. 54. Colonies in Flask.
(*After Frankland.*)

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[1] *Philosophical Transactions*, vol. clxxviii. p. 113.

containing two small porous plugs placed one in front of the other. The first plug consists of glass-wool coated with sugar, whilst the second contains, in addition, a layer, ⅛ inch in thickness, of fine sugar-powder. The microbes, suspended in the aspirated air, are deposited on these plugs, which are introduced into separate flasks, each containing a a small quantity of melted nutrient gelatine. Each flask is then agitated until the plug is disintegrated, and since the sugar-coating of the glass-wool dissolves in the liquid gelatine, the microbes become immediately detached. The gelatine is now allowed to solidify, forming a thin film over the inner surfaces of the flasks. The flasks are finally placed in an incubator; and in a few days colonies derived from the microbes, which were collected by the plugs make their appearance and can be counted and further studied (Fig. 54).

The author[1] has examined the air of Lincoln, Paris, and London. The methods used for estimating the number of microbian colonies in a known volume of air were those of Hesse and Frankland. Before August 6th, 1888, Hesse's method was used, while after that date Frankland's method was substituted for that of Hesse. The average number of colonies in three gallons (fifteen litres) of air are given in the following tables:—

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[1] *Proceedings of Royal Society of Edinburgh*, vol. xvii. p. 265; and *Researches on Micro-Organisms*, p. 59.

THE AIR OF LINCOLN.

PLACE.	YEAR 1887.											
	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.
(1) Top of hill (near Cathedral),	3	6	14	16	19	25	34	—	30	28	12	4
(2) Base of hill (Broadgate), .	18	26	30	41	50	62	65	—	59	57	19	17

THE AIR OF PARIS.

Place or Part of Paris.	Situation in Paris.	August, 1887.
(1) Cimetière du Père la Chaise,	E.	96
(2) Boulevard Saint-Germain, .	Centre	104
(3) Forest of Ville d'Avray, .	S.W.	81
(4) Rue de Rennes, . . .	Centre	99
(5) Palais du Trocadéro, . .	W.	50
(6) Park of Versailles, . .	S.W.	78
(7) St. Cloud, , . . .	S.W.	82
(8) Boulevard Voltaire, . .	E.	100
(9) Cimetière Montparnasse, .	S.	98
(10) Cimetière Montmartre, .	N.	95
(11) Parc des Buttes Chaumont, .	N.E.	80

THE AIR OF LONDON.

Place or Part of London.	July, 1888.	August, 1888.
(1) Forest Gate (Essex). . .	64	79
(2) City (near Bank), . . .	85	110
(3) West End (Piccadilly), . .	80	96
(4) East End (near Mint), . .	88	106

The conclusion drawn from these investigations are the following:—

(*a*) There are a larger number of microbes in the atmosphere during the summer than either the spring or winter. They appear to reach a maximum during the month of August. (*b*) The number of microbes found in the atmosphere decreases, the higher one ascends. Hence near the Lincoln Cathedral there are fewer microbes in the atmosphere (on any given day) than in the valley of the Witham. The same remark also applies to the number of microbes found in the atmosphere at the top of the Trocadéro Palace, Paris, where there are fewer microbes than in a low-lying but crowded thoroughfare like the Boulevard Saint-Germain. (*c*) There are a larger number of microbes in the atmosphere of crowded centres than in less densely-populated districts. (*d*) By gradually passing from a large city towards the country the number of aërial microbes decreases; *e.g.*, there are fewer microbes in the atmosphere of the Forest of Ville d'Avray, the Park of Versailles, and the village of St. Cloud, than in the principal thoroughfares of Paris and London.

Dr. P. Miquel[1] (who is the greatest authority on aërial microbes) has published elaborate tables concerning the number of microbes in the air of certain parts of Paris. During the year 1888, Miquel obtained the following mean number of microbes in the air (per cubic meter) at Montsouris, and in the vicinity of the Hôtel de Ville, Paris:—

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[1] *Annuaire de l'Observatoire de Montsouris*, 1877-92.

SEASONS.	Montsouris.	Hotel de Ville.
Winter, ...	171	2870
Spring, ...	210	8920
Summer, ...	400	12280
Autumn, ...	185	6800
Annual Means, . . .	242	7720

The mean annual results (for eight years, 1881-88) of the number of microbes contained in one cubic metre of the air at Montsouris and in the vicinity of the Hôtel de Ville (*i.e.* in the *centre* of Paris) are given in the following table:—

	Montsouris.	Hotel de Ville.
January, ...	228	2310
February, ...	170	3140
March, ...	255	3420
April, ...	358	4340
May, ...	379	5950
June, ...	448	5070
July, ...	676	5200
August, ...	628	5640
September, ...	470	5510
October, ...	332	4335
November, ...	239	3700
December, ...	189	2885

From the above results it will be seen (*a*) that there are a larger number of microbes in the atmosphere in the centre of Paris than at Montsouris;

(*b*) that there are a larger number of microbes in the atmosphere during the summer than any other period of the year. Miquel has also shown that as the number of microbes in the atmosphere increases, so does the mortality from zymotic or infectious diseases.

The investigations of Dr. P. F. Frankland on aërial microbes have added considerably to our knowledge of this interesting subject. Frankland has not only examined the air so as to ascertain the number of microbes present in a known volume, but he has discovered many new forms.[1]

Frankland has obtained the following results concerning the number of microbes present in ten litres, or two gallons, of air at different places:—

Different altitudes.	Primrose Hill (top),	9
	,, ,, (bottom),	24
	Norwich Cathedral (top of spire, 300 ft.),	7
	,, ,, (tower, 180 ft.),	9
	,, ,, (in the close),	18
	St. Paul's Cathedral (Golden Gallery),	11
	,, ,, (Stone Gallery),	34
	,, ,, (Churchyard),	70
Country Places.	Reigate Hill,	2
	Heath near Norwich,	7
	Garden at Reigate,	25
	Garden near Norwich,	31
Open Places in London.	Kensington Gardens,	13
	Hyde Park,	18
	Exhibition Road,	554

Frankland has also shown that within doors the number of microbes suspended in the air depends upon the number of people present and the amount of disturbance of the air which is taking place.

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[1] *Philosophical Transactions*, vol. clxxviii. p. 257.

Dr. Fischer[1] has proved that sea air is almost free from microbes. Carnelley[2] and Pétri[3] have also shown that the air of sewers is remarkably free from microbes. This is due to the moisture on the walls of these subterranean channels.

The following microbes are always present (more or less) in the atmosphere:—

Micrococcus citreus conglomeratus.
Micrococcus violaceus.
Micrococcus rosaceus.
Bacterium indicum.
Micrococcus prodigiosus.
Bacterium aceti.
Bacterium lactis.
Micrococcus cyaneus.
Bacterium xanthinum.
Bacillus figurans.
Micrococcus carnicolor.
Micrococcus candicans.
Micrococcus albus.
Sarcina lutea.
Sarcina aurantica.
Bacillus fluorescus.
Micrococcus liquefaciens.
Sarcina liquefaciens.
Micrococcus gigas.
Micrococcus chryseus.
Bacillus aurescens.
Bacillus aureus.
Bacillus citreus.
Bacillus plicatus.
Bacillus chlorinus.
Bacillus polymorphus.
Bacillus profusus.
Bacillus pestifer.
Bacillus lævis.
Bacillus cereus.
Bacillus subtilis.

Besides other microbes, there are always present in the atmosphere an abundance of moulds and yeast-fungi.

Although the microbes connected with the common infectious diseases have not been discovered in air, 'yet there can be no doubt that, in the immediate vicinity of the foci of disease, such microbes are present, and that their distribution and conveyance in the air will take place in just the same manner as in the case of non-pathogenic microbes. The investigations on aërial microbia, so far as they have as yet been carried, are of service in indicating how we may escape from all microbes, whether harmful or harmless; and secondly, how we may avoid the conveyance of microbes into the atmosphere from places where pathogenic forms are known or likely to be present. This acquaintance with the distribution of microbes in general, and the power of controlling their dissemination which it confers, is really of far wider practical importance than discovering whether some particular pathogenic form is present in some particular sample of air. It is this knowledge which has led to the vast improvements in the construction and arrangement of hospital wards and of sick-rooms generally, and which has directed attention to the importance of avoiding all circumstances tending to disturb and distribute dust. It is, moreover, this knowledge of the distribution of microbes in our surroundings which has formed one of the foundations for the antiseptic treatment of wounds—that great step in surgery with which the name of Sir Joseph Lister is associated.'[1]

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[1] *Zeitschrift für Hygiene*, vol. i.
[2] *Philosophical Transactions*, vol. clxxviii. p. 61.
[3] *Zeitschrift für Hygiene*, vol. iii.

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[1] For further information see Frankland's papers in *Journal of Society of Arts*, vol. xxxv. p. 485; *Proc. Roy. Soc.*, 1885-86; Miquel's *Les Organismes Vivants de l'Atmosphère*; Prudden's *Dust and its Dangers*; and Griffiths' *Researches on Micro-Organisms.*

CHAPTER VIII

THE MICROBES OF THE SOIL

SOIL is very rich in microbes, and these insignificant plants play a most important part in the processes of putrefaction and nitrification, which are always at work for man's benefit and welfare.

Among the microbes present (more or less) in soil are the following:—

The Nitrous Bacillus.
The Nitric Micrococcus.
Bacillus tardecrescens.
Bacterium ureæ.
3 *Bacillus fluorescens.*
Micrococcus cereus.
Bacillus of Mouse Septicæmia.
Bacillus mycoides.
Bacillus anthracis.
Bacillus of tetanus.
Bacillus of malariæ.
Spirillum choleræ Asiaticæ.
Bacillus typhosus.
Bacillus radicicola.
Bacterium septicum agrigenum.
Bacillus œdematis maligni.
Streptococcus septicus.
Bacillus subtilis.
Bacillus toruliformis.
Bacillus floccus.
Bacillus septicus.
Bacterium termo.
Bacterium allii (?).

In addition to the above, the spores, etc., of many of the higher fungi are present in soil. Some of these are detrimental to the growth of vegetation, for they become internal or external parasites, and thereby produce disease.[1] Not only are the higher plants attacked by parasites present in soil, but man and animals suffer from diseases, like tetanus, malaria, etc., which are caused by soil-microbes.

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[1] Griffiths' *Diseases of Crops.*

To study the microbes present in soil, both solid and liquid media are used, but the employment of the former is much more satisfactory. The following methods are used by bacteriologists to ascertain the number of microbes in a known weight, etc., of soil:—(*a*) A sample of the dried soil is triturated with sterilised distilled water, and then a small quantity of this water is sprinkled on the surface of a gelatine plate. (*b*) The soil is introduced into a test-tube containing liquefied gelatine. After a thorough shaking the mixture is poured out upon a glass plate, so as to form a plate-cultivation. (*c*) When bouillon is used the soil is first triturated with water, and then a drop of the water is transferred to a flask containing sterilised bouillon.

Among the results obtained of the number of microbes present in various soils are the following:

(A) GRIFFITHS' ANALYSES.

Samples of Soil from—	Number of Microbes in 1 gram.
Lincoln (Monk's Road),	611,000
,, (Castle grounds),	720,000
Manchester (Infirmary grounds),	1,230,000
,, (Plymouth Grove),	550,000
London (Forest Gate),	430,000
,, (Hyde Park),	820,000
Paris (Forest of Ville d'Avray),	780,000
,, (Near Sèvres),	880,000
,, (Parc Monceaux),	754,000
Dieppe (near Church of St. Jacques),	1,360,000
,, (near the Casino),	1,200,000
New Zealand (after 14 weeks' desiccation),	240,000

(B) MIQUEL'S ANALYSES.

Samples of Soil from—	Number of Microbes in 1 gram.
Paris (Rue de Rennes), ...	2,100,000
,, (Rue de Monge), ...	1,300,000
,, (Parc du Montsouris), ...	750,000

Dr. C. N. Dowd[1] has recently ascertained the number of microbes in soil derived from various streets in New York. The soil in each case was obtained during the upturning of the streets for relaying gas and water-pipes, etc.

(C) DOWD'S ANALYSES.

Samples of Soil from—	Number of Microbes per cc.
East Fifty-ninth Street, near Third Avenue, .	17,675
East Fifty-nine Street, near Park Avenue, .	17,950
East Fifty-ninth Street, near Fifth Avenue, .	157,200
East Fifty-ninth Street, near Madison Avenue,	131,100
Eighth Avenue and Fifty-seventh Street, . .	29,700
Tenth Avenue and Sixty-fifth Street, . . .	29,250
Eighth Avenue, near Fifty-sixth Street, . .	8585
Eighth Avenue, near Fifty-fifth Street, . .	3800
Fifty-ninth Street and Sixth Avenue, . . .	10,650
Fiftieth Street and Eighth Avenue, ...	287
Sixth Avenue, near Fifty-eighth Street, . .	33,150
Seventh Avenue and Fifty-fourth Street, . .	15,250
Seventy-first Street, near Eighth Avenue, . .	20,150
Forty-ninth Street and Eleventh Avenue, . .	24,900
Third Avenue, near Forty-second Street, . .	28,850
Third Avenue, near Forty-second Street, . .	67,500

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[1] *American Medical Record*, 1890.

To ascertain the number of microbes in a given sample of soil, the colonies produced on the gelatine-plates are accurately counted. This is performed by the apparatus represented in Fig. 55, which will be described in the next chapter. To ascertain the characteristics of the microbes, further cultivations must be made; the microbes must be transplanted into various media, and exposed to different temperatures; and they must be inoculated into different kinds of animals. The labour of separating each species and studying it in detail would be extremely great; hence microbian soil examinations have largely been confined to the determination of the number of microbes, and not to the peculiar species. In determining the significance of such examinations, we must bear in mind the following facts:—(1) The number of microbes present in a soil does not necessarily indicate the number of pathogenic forms. (2) Small samples of soil may show marked variations in the number of microbes; this is owing to minor local influences. (3) Surface soil always contains a larger number of

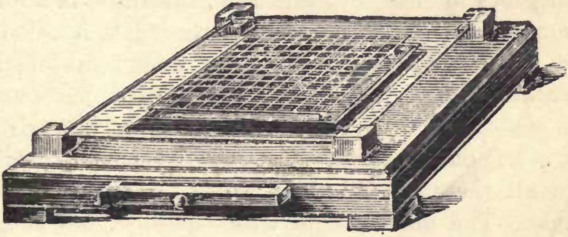

Fig. 55. Wolffhügel's Apparatus.
(For estimating the number of Colonies in a Plate-cultivation.)

microbes than sub-soil; and at a depth of 8 or 10 feet there are hardly any present. (4) Most of the microbes of soil are harmless when introduced into the human or animal body; but the bacilli of tetanus, anthrax,[1] typhoid fever, malaria, and cholera have been found in soil.

Dowd's investigations have proved that the exposure of so much soil in the upturning of streets is detrimental to the health of the surrounding community. However, it should be remembered that so long as the soil is wet it cannot spread the microbes in the air; but the soil does not long remain wet. It dries beside the trenches, it adheres to the implements, the clothes and boots of the workmen, and, in fact, to everything which comes in contact with the trenches; and, finally, much of it is left on the surface when the pavement is relaid. In all these conditions it may be carried away as dust. The microbes go with the dust, and access to the body is then made easy. The amount of dust in the air is much increased by these trenches; but, on the other hand, the deeper layers of soil or earth from which this dust is derived do not contain nearly so many microbes as the surface layer.

An important method for dealing with the dust of streets, especially during epidemics, is to water them with some germicidal substance, by means of the Strawsonizer or pneumatic distributor.[2] This machine is capable of distributing one or more gallons of any fluid over an acre of land, and to a width of 23 feet. Therefore, it would be advantageous to use this machine for watering streets, cattle markets, etc., with a weak solution of 'sanitas,' carbolic acid, or any other cheap disinfectant.

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[1] Pasteur in *Bulletin de l'Académie de Médecine*, 1880.

[2] Obtainable at Messrs. Strawson and Co., Newbury, Berkshire.

Concerning *cultivated* soils, nitrogen is a most important element in the growth of crops. Berthelot[1] has shown that a fixation of atmospheric nitrogen takes place in certain vegetable soils by the action of microbes and other fungi.

Hellriegel and Wilfrath have proved that leguminous plants obtain their great supplies of nitrogen from the air. This power of absorbing free nitrogen is due to the roots of leguminous plants becoming inoculated with the microbes present in soil. The microbes, which give rise to tubercles on the roots and rootlets, enter into a partnership or symbiotic relationship with the leguminous plant for mutual advantage. These microbes have the power of bringing the free nitrogen into organic combination.

Perhaps the chief soil-microbe which enters into symbiosis with leguminous plants is Dr Beyerinck's *Bacillus radicicola.* This microbe has been isolated from cultivated soils as well as from the tubercles on the roots of *Vicia faba* (the field bean); and Beyerinck has inoculated the roots of seedling-beans with this microbe, and in each case it multiplied within the roots, ultimately giving rise to tubercles.

The process of *nitrification* or the conversion of organic and ammoniacal nitrogen into nitrates was first shown by Müntz and Schloësing[1] to be due to the action of microbes in the soil. Although these *savants* had previously described a microbe causing nitrification, it was not until 1890 that Dr. P. F. Frankland, F.R.S.,[2] M. Winogradsky,[3] and Mr. R. Warington, F.R.S.,[4] simultaneously described the *true* cause of nitrification. The nitrifying microbes were isolated by the fractional dilution method.

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[1] *Comptes Rendus*, vol. cviii.

(1) *Frankland's researches.*—Dr. and Mrs. Frankland have isolated a nitrifying microbe from soil. 'Nitrification having been in the first instance induced in a particular ammoniacal solution by means of a small quantity of garden soil, was carried on through twenty-four generations, a minute quantity on the point of a sterilised needle being introduced from one nitrifying solution to the other. From several of these generations gelatine-plates were poured, and the resulting colonies inoculated into identical ammoniacal solutions, to see if nitrification would ensue; but although these experiments were repeated many times, on no occasion were they successful.' In other words, the microbe in question refused to grow on gelatine. The ammoniacal solution, already referred to, contained:—1000 cc. of distilled water, 100 cc. of salt solution,[5] 0·5

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[1] *Comptes Rendus*, vol. xlviii. p. 301; vol. lxxxv. p. 1018; vol. lxxxix. pp. 891 and 1074.

[2] *Philosophical Transactions*, vol. clxxxi. pp. 107-128.

[3] *Annales de l'Institut Pasteur*, 1890, p. 213 *seq.*

[4] *Journal of Chemical Society*, 1891, pp. 484-529; *Chemical News*, vol. lxi. (1890), p. 135.

[5] This solution contained 1 gramme of potassium phosphate, 0·2 gramme of crystallised magnesium sulphate, and 0·1 gramme of calcium chloride (fused) in 1000 cc. of water.

gramme of ammonium chloride, and 5 grammes of carbonate of lime (pure); and in this solution the microbe grew and multiplied. As this solution contains no organic matter, it will be seen that nitrification can take place in purely mineral solutions. This power of growing in mineral solutions prevented the development of other microbes (present in the soil used for inoculation) which require organic matter for their growth. After proving that the microbe refused to grow on gelatine, 'experiments were commenced to endeavour to isolate the microbe by the dilution method. For this purpose a number of series of dilutions were made by the addition, to sterilised distilled water, of a very small quantity of an ammoniacal solution which had nitrified. It was hoped that the attenuation would be so perfect that ultimately the nitrifying microbe alone would be introduced. After a very large number of experiments had been made in this direction, the authors at length succeeded in obtaining an attenuation consisting of about one-millionth of the original nitrifying solution employed, which not only nitrified,[1] but, on inoculation into gelatine-peptone, refused to grow, and was seen, under the microscope, to consist of numerous characteristic bacilli hardly longer than broad, which may be described as bacillococci.'

The chief characters of the Frankland *Bacillus of nitrification* (Fig. 56 A) are the following:—

(*a*) The solutions in which the isolated microbe grows remain perfectly clear. (*b*) The microbe has the remarkable capacity of indefinite growth in a medium devoid of organic matter. (*c*) It is 0·8 μ in length, and hardly longer than broad, hence it has been called a bacillococcus. It occurs both isolated, in pairs, and in small irregular groups. (*d*) In the living state it exhibits a vibratory movement only. (*e*) The microbe cultivated in ammoniacal solutions converts the ammoniacal into *nitrous* nitrogen, and not into nitric nitrogen. (*f*) The same microbe appears to grow in broth or bouillon, but not on solid gelatine-peptone.

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[1] The presence of nitrous acid was ascertained by both diphenylamine and sulphanilic acid.

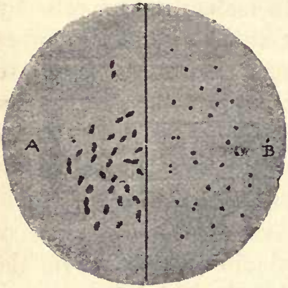

FIG. 56. MICROBES OF NITRIFICATION.
A, Frankland's nitrous bacillus.
B, Warington's nitric micrococcus.

(2) *Winogradsky's researches.*— Winogradsky has also obtained a similar bacillus to that of Frankland, which grows in an inorganic ammoniacal solution, but not on gelatine-peptone; and he has shown that this microbe grows (and may be isolated) on the surface of gelatinous silica containing the inorganic ammoniacal salts already mentioned. This nitrifying microbe gives rise to very characteristic colonies on gelatinous silica. Winogradsky's bacillus measures from 1·1 to 1·8 μ long, and does not exceed 1 μ broad. This microbe occurs singly, in pairs, rarely in chains of three to four individuals, and as zooglœa. It converts ammoniacal into

nitrous nitrogen, and can grow in ammoniacal solutions devoid of organic matter. There is little doubt that Frankland's and Winogradsky's microbes are the same. Both sets of experiments prove that the nitrous bacillus of the soil converts ammoniacal into nitrous nitrogen, and not into nitric nitrogen.

(3) *Warington's researches.*—Mr. Warington has also isolated, by the dilution method, a microbe which converts ammonia into nitrous acid only; and confirms the investigations of Frankland and Winogradsky. In addition to this, Warington has apparently isolated a microbe from soil which converts nitrites into nitrates. This microbe produces neither nitrites nor nitrates in ammoniacal solutions; in fact, it cannot oxidise ammonia. The nitric microbe (Fig. 56 B) is a micrococcus, and grows in a solution of potassium nitrite.

'The nitrification effected by soil is thus explained as performed by two microbes, one of which oxidises ammonia to nitrates, while the other oxidises nitrites to nitrates. The first microbe is easily separated from the second by successive cultivations in solutions of ammonium carbonate. The second is (probably) separated as easily from the first by successive cultivations in solutions of potassium nitrite containing monosodium carbonate.'

'In soil the nitric microbe is equally active as the nitrous, since soil never contains any but extremely weak solutions of ammonia, and supercarbonates are always present.'

LIBRARY OF THE UNIVERSITY

CHAPTER IX

THE MICROBES OF WATER

THE organisms present in water have long been observed by the aid of the microscope, but it is only during the last decade that bacteriological methods have been introduced for the systematic examination of potable and other waters.

Water is one of the most convenient vehicles for the distribution of microbes, and unfiltered water abounds in these small specks of animated matter. This need not cause any surprise, because, as we have already seen, the atmosphere and soils are laden with microbes. In fact, every shower of rain diminishes the number of microbes suspended in air. These are then found in puddles, pools, ponds, rivers, etc., and consequently are carried into well and other potable waters. Although the majority of these microbes are harmless, it is always advisable to filter water before use. One of the best filters for this purpose is Maignen's 'Filtre Rapide.' Among the various microbes found in water are the following:—

Fresh Water (polluted or otherwise).	Sea Water.	Pond Water (marshes, etc.).	Brackish Water.	In Special Cases.
Bacterium termo.	*Sarcina litoralis.*	*Sarcina hyalina.*	*Spirillum violaceum.*	*Bacillus typhosus.*
Bacillus aquatilis.	*Bacterium litoreum.*	*Bacterium lineola.*	*Spirillum Rosenbergii.*	*Spirillum choleræ Asiaticæ.*
Bacillus aborescens.	*Bacterium fusiforme.*	*Spirochæta plicatilis.*		*Bacillus malariæ.*
Bacillus liquidus.	*Spirochæte gigantea.*	*Spirillum rugula.*		*Bacillus anthracis.*
Bacillus vermicularis.	*Spirillum attenuatum.*	*Spirillum undula.*		*Bacillus of tetanus.*
Bacillus nubilus.	*Beggiatoa mirabilis.*	*Spirillum sanguineum.*		
Bacillus ramosus.	*Phragmidiothrix multiseptata.*	*Spirillum volutans.*		
Bacillus aurantiacus.	*Bacterium Fischeri.*	*Sarcina Reitenbachi.*		
Bacillus viscosus.	*Bacterium balticum.*	*Beggiatoa alba.*		
Bacillus violaceus.	*Bacterium Indicum.*	*Beggiatoa roseo-persicina.*		
Bacillus diffusus.	*Bacterium luminosum.*	*Sphræotilus natans.*		
Bacillus candicans.		*Spiromonas volubilis.*		
Bacillus scissus.		*Spiromonas Cohnii.*		
Bacillus erythrosporus.				
Bacillus subtilis.				
Bacillus ulna.				
Bacterium merismopedioides.				
Bacillus ianthinus.				
Proteus vulgaris.				
Crenothrix kühniana.				
Cladothrix dichotoma.				
Bacterium xanthium.				
M. citreus. conglomeratus.				
Micrococcus chlorinus.				
Sarcina ventricula.				
Bacillus pyocyaneus.				
Spirillum tyrogenum.				
Sarcina lutea.				
Bacterium cavicida.				
Bacterium hyacinthi.				

In addition to the *Schizomycetes*, various *Protozoa*, etc., are always present (more or less) in water, and Fig. 57 represents certain animal and vegetal forms found in some potable waters.

Although the majority of *Schizomycetes* and *Protozoa* found in waters are harmless, it has been proved that certain outbreaks of typhoid fever, cholera, etc., have been traced to water supplies; and the microbes of typhoid fever, cholera, tetanus, etc., have all been found in drinking waters contaminated with sewage. Dysentery and tropical abscess of the liver are due to certain species of *Amœbæ*,

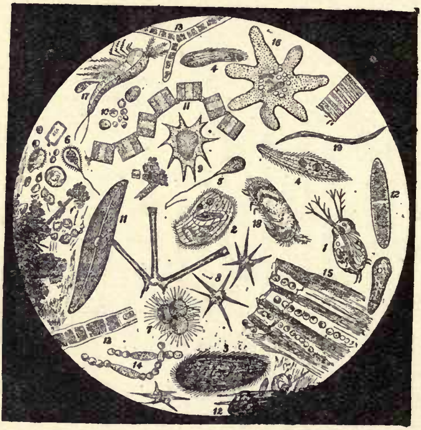

Fig. 57. Infusoria, etc., in Water.

1, Daphnia. 2, Chilodon. 3, Paramœcium. 4, Acineria. 5, Paranema. 6, Cercomonas. 7, Actinophrys. 8, Amœbæ. 9, Amœba diffluens. 10, Protococcus. 11, Diatoms. 12, Desmids 13, Confervæ. 14, Spores of fungi. 15, Pieces of vegetable tissue. 16, Amœba (more highly magnified). 17, Cyclops. 18, Cypris. 19, Anguillula.

which enter the system through the medium of water. In view of these facts the bacteriological analysis of water is a subject of great importance; but the primary object in such analyses is *not* the search for pathogenic microbes. Such an investigation is generally fraught with insuperable difficulties, and, for sanitary purposes, is practically worthless. 'It is obvious that, even if the typhoid bacillus, or any other pathogenic microbe could be detected with unerring certainty in any water in which it was present, a search for this bacillus in the ordinary course of water examination would still have only a very subsidiary interest. Waters are surely not only to be condemned for drinking purposes when they contain the germs of zymotic disease at the time of analysis, but in all cases when they are subject to contaminations which may at any time contain such germs. Sewage-contaminated waters must on this account be invariably proscribed, quite irrespectively of whether the sewage is, at the time that the water is submitted to examination, derived from healthy or from diseased persons. . . . The real value of these bacteriological investigations, if judiciously applied, consists in their power of furnishing us with information as to the probable fate of dangerous organisms, should they gain access to drinking water. It is by their means that we have learnt that many such organisms can preserve their vitality, nay, in some cases can actually undergo multiplication in ordinary drinking water; that they are destroyed by maintaining the water at the boiling point for a short time; and that they are more or less perfectly removed by some processes of filtration and precipitation, whilst other processes of the same nature are worthless, or even worse' (Frankland).

Before describing the methods for the bacteriological examination of waters, we must allude to (*a*) the collection of the samples, and (*b*) the transport of the same.

To collect the samples of water accurately stoppered bottles (70 cc. capacity) are used. These must be perfectly clean, and rinsed out with distilled water. Each bottle is put into a small tin canister, and the canisters (containing the bottles) are heated in a steriliser to about 180° C. for at least three hours. 'The bottles thus sterilised can be easily transported without suffering contamination by dust to the place where the sample is to be collected. In collecting the sample of water the outside of the bottle should be rinsed in the water before removing the stopper, and when the bottle is opened the water is at once allowed to enter and fill the bottle to the extent of four-fifths, the stopper being immediately replaced and tightly screwed in, so that the exposure to the air is reduced to a minimum. The bottle is replaced in the tin canister, and the lid closed. In collecting samples of water from rivers, reservoirs, lakes, or ponds, it is better not to remove the stopper until the bottle is completely immersed in the water, and to replace it while still beneath the surface.' After collection the sample of water should be examined as soon as possible, for it has been proved by Dr. T. Leone,[1] Dr. P. F. Frankland,[2] and others, that microbes multiply very rapidly in water. For instance, Leone gives the following figures, which show the rapid increase of microbes in a sample of water kept for only five days :—

Number of Microbes in 1 *cc. of water* (*at* 14° *to* 18° *C.*).

Water on day of collection,	5
,, after 1 day's standing	100
,, ,, 2 days' ,,	10,500
,, ,, 3 ,, ,,	67,000
,, ,, 4 ,, ,,	315,000
,, ,, 5 ,, ,,	500,000

If the water has to be transmitted a considerable distance, occupying several days in transit, Dr. P. Miquel[3] recommends the use of a *glacière*, or box, in which the bottle is surrounded with ice.

There are two principal methods in use for the bacteriological examination of water. The first is the plate-cultivation process, which consists in taking a known quantity (say 1 cc.) of the water, and mixing it with melted nutrient gelatine contained in a test-tube. After shaking, the contents of the tube are rapidly poured out upon a sterilised glass plate, then allowed to solidify, and finally placed in a damp chamber, kept at about 22° C. After a few days' incubation colonies make their appearance on and in the layer of gelatine. The colonies are counted by means of the eye or lens, with the aid of Wolffhügel's counting apparatus (see Fig. 55), which consists of a glass plate, ruled with vertical and horizontal lines into centimetre squares, which are often sub-divided. The cultivation-plate is placed on a black background, and the ruled glass plate placed over the former, without touching the colonies. 'If the colonies are very numerous the number in some small divisions is counted; if less, in some large ones; and an average is obtained from which the number of colonies on the entire surface is calculated.'

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[1] *Gazzetta Chimica Italiana*, vol. xv. (1885), p. 385.

[2] *Proceedings of Royal Society*, 1886.

[3] *Manuel Pratique d'Analyse Bactériologique des Eaux* (1891), p. 26.

The second method is largely used in France, and is known as 'fractionnement dans le bouillon.' The sample is first diluted with sterilised water of known volume. After this one gramme (1 cc.) of the water is taken up by means of a sterilised capillary pipette, which is dipped four times into the water at different points of the liquid mass to obtain the above-mentioned quantity. By this means a fair sample of the water is obtained. In the laboratory of Dr. P. Miquel thirty-six small flasks (each 15 cc. capacity) are each half filled with sterilised bouillon. These flasks, having each a glass cap containing a sterilised cotton-wool plug, are placed in a divided box. Each flask receives one, two, or three drops of the sample of water, as the case may be; all the flasks are placed in an incubator at 30°-35° C. during a period of at least fifteen days, when the microbian colonies are counted.

Before introducing the small quantity of water into either a solid or a liquid medium, the original sample should be violently shaken to ensure an even distribution of the microbes throughout the water.

By using Koch's or the plate-cultivation method, the author[1] obtained the following average number of microbes (colonies) in 1 cc. of a sample of water from the river Witham (at Lincoln) during the year 1887:—

January,	2,016	July,	10,184
February,	3,488	August,	—
March,	10,287	September,	4,110
April,	11,692	October,	9,621
May,	11,923	November,	10,211
June,	12,000	December,	9,787

These figures (monthly means) give a yearly mean of 8665 microbes in 1 cc., or quarterly means as follows:

Spring,	11,300
Summer,	11,092
Autumn,	7,980
Winter,	5,097

From these results the greater number of microbes in the Witham were during the spring and summer. Another series of experiments with water from certain rivers gave the following results:—Witham, 11,860; Irwell, 9230; Thames, 25,745; and the Seine, 56,219 microbes per cubic centimetre.

Dr. P. F. Frankland[2] has made periodical examinations of the river and well waters from which the water-supply of London is derived; and during the year 1886, he obtained the following number of colonies (on gelatine-plates) per 1 cc. of water:—

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[1] Griffiths' *Researches on Micro-Organisms*, p. 77.

[2] *Journal of Society of Chemical Industry*, vol. iv. (1885), and vol. vi. (1887); *Transactions of Sanitary Institute*, vols. viii. and ix.; *Proc. Roy. Soc.*, 1885; and *Proc. Inst. of Civil Engineers*, 1886.

DESCRIPTION OF WATER (London Companies, etc.)	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Average.
THAMES.													
Thames unfiltered, .	45,400	15,800	11,415	12,250	4,800	8,300	3,000	6,100	8,400	8,600	56,000	63,000	20,255
Chelsea, . . .	159	305	299	94	59	60	59	303	87	34	65	222	146
West Middlesex, . .	180	80	175	47	19	145	45	25	27	22	47	2,000	234
Southwark, . . .	2,270	284	1,562	77	29	94	380	60	49	61	321	1,100	524
Grand Junction, . .	4,891	208	379	115	51	17	14	12	17	77	80	1,700	630
Lambeth, . . .	2,587	265	287	209	136	129	155	1,415	59	45	108	305	475
LEA.													
Lea unfiltered, . .	39,300	20,600	9,025	7,300	2,950	4,700	5,400	4,300	3,700	6,400	12,700	121,000	19,781
New River, . . .	363	74	95	60	22	53	46	55	17	10	32	400	102
East London, . .	224	252	533	269	143	445	134	243	165	97	248	280	253
DEEP WELLS.													
Kent (well direct), .	—	5	44	7	8	4	12	9	5	82	12	11	18
Kent (district), . .	43	149	38	47	101	39	48	13	25	344	196	66	92

It will be seen from the above analyses that the number of microbes is greatly reduced by the methods of filtration, etc., in use by the various London water companies. The average reduction in the number of developable microbes present in the river waters before delivery by the companies is from 96·2 to 99·1 per cent.

Dr. Miquel[1] has also made periodical examinations of the various waters in and around Paris. Paris takes its supplies of water from the Seine and Marne. There are three water-works belonging to the former, and one belonging to the latter river. During 1890 Miquel obtained the following results with the Paris water supply :—

Months.	Seine.			Marne.
	Ivry.	Austerlitz.	Chaillot.	St. Maur.
January,	52,670	41,020	85,350	75,960
February,	43,620	59,590	107,590	58,120
March,	34,710	46,070	80,920	57,750
April,	38,640	29,020	86,760	16,310
May,	12,930	30,960	37,920	12,890
June,	28,150	40,340	90,860	14,270
July,	14,130	26,830	84,520	10,450
August,	6,780	21,910	121,430	13,570
September,	20,220	76,170	227,400	6,410
October,	22,350	42,390	143,120	11,860
November,	37,720	45,690	144,200	95,590
December,	78,950	73,820	129,900	62,470
Yearly means,	32,530	44,490	111,660	36,305

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[1] *Annuaire de l'Observatoire de Montsouris*, 1887-91.

The above results represent the monthly means of the number of microbes (colonies) obtained from 1 cc. of water by the 'fractionnement' method.

The quarterly means are given in the next table:—

	SEINE.			MARNE.
	Ivry.	Austerlitz.	Chaillot.	St. Maur.
Winter, .	43,500	48,890	91,285	63,940
Spring, .	26,570	33,440	71,845	14,490
Summer, .	13,710	41,635	144,250	10,140
Autumn, .	46,340	53,965	139,070	56,640

From Miquel's analyses the quarterly means of the number of microbes contained in 1 cc. of sewer-water (collected at Clichy and St. Ouen, Paris) are as follows:—

Winter, ...	14,780,000
Spring, ...	16,760,000
Summer, ...	9,638,000
Autumn, ...	6,375,000

It appears from the observations of Miquel that the largest number of microbes found in river and sewer waters is during the spring months. The self-purification of rivers polluted with sewage has given rise to a great deal of discussion; but there is no doubt that in some rivers the sewage is rapidly oxidised by the oxygen dissolved in the water or separated from plants. Professor von Pettenkofer[1] states that sewage may be permitted to flow into a river if its volume is not more than $\frac{1}{15}$th that of the river water and its rate of flow decidedly greater than that of the current; and it has been shown that the Isar, for example, possesses this self-purification. The bacteriological investigations of Prausnitz prove the purifying power of the Isar. The number of 198,000 microbes per cc. found at the mouth of the Munich sewer was reduced at Ismaning to 15,231, and at Freising to 3602. These results agree with those of other bacteriologists. Fraënkel found in the water of the Spree above and below Berlin 6000 microbes per cc., but in the city a million. It has been stated that 'the mere number of microbes found has, however, no sanitary significance, since the microbes found in the water are almost exclusively harmless, and, indeed, destroy the pathogenic microbes in the struggle for existence.' But it should not be forgotten that Grüber[1] and Frankland[2] have shown that *Spirillum choleræ Asiaticæ*, *Bacillus anthracis*, etc., are capable of living and multiplying in sewage, and that the first-named microbe retained its vitality for 11 months 'in company with countless numbers of a micrococcus which had accidentally gained access.'

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[1] *Chemiker Zeitung*, 1891.

'It is necessary, therefore, to exercise considerable caution in judging upon this point in the present state of our knowledge, and it would be highly premature to place too much reliance upon this alleged destruction of pathogenic forms by non-pathogenic ones.' With all due respect to such an authority as Von Pettenkofer, 'we learn that ordinary sewage forms a suitable medium not only for the indefinite preservation of some pathogenic microbes, but also, in some cases, for their rapid growth and multiplication.'

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[1] *Wiener Medicinische Wochenschrift*, 1887.

[2] *Proceedings of Royal Society*, 1886; and *Journ. Society of Chemical Industry*, vol. vi. (1887).

It is well known that surface waters (*e.g.* rivers, ponds, etc.) are rich in microbial life; but waters, derived from deep wells and springs, which have undergone natural filtration through porous strata, contain only few microbes. Frankland has shown that 'this removal of microbes from water also takes place in a very marked manner when it is submitted to some kinds of artificial filtration, such as that through very finely-divided coke or charcoal, as well as in the filtration of water on the large scale through sand.' A glance at Frankland's table (p. 294) shows the great reduction in the number of microbes present in the water obtained from the Thames and the Lea, after filtration through fine sand. But, according to Frankland, the following factors are calculated to influence the number of microbes present in the distributed water:—

(*a*) Storage capacity for unfiltered water.
(*b*) Thickness of fine sand used in filtration.
(*c*) Rate of filtration.
(*d*) Renewal of filter-beds.

(*a*) Through greater storage capacity, the necessity of drawing the worst water from the river is avoided, a matter which in the case of a stream like the Thames, liable to frequent floods, is of great importance. During the period of storage the water deposits the greater part of its suspended matter, including a large proportion of the microbes. Then a further diminution takes place through degeneration and decay of the microbes, for the number of microbes in the unfiltered river-waters diminishes on keeping irrespectively of subsidence, probably owing to the competition between different forms hostile to each other, as well as by the production of chemical compounds inimical to their further multiplication. (*b*) That the thickness of the filtering stratum should exercise an important influence on the number of microbes passing through the filter must be sufficiently obvious. In estimating the thickness of such a sand filter the fine sand only should be taken into consideration, as it is only this portion of the filter which can have any effect in the removal of microbes. (*c*) That the filtration is the more perfect the slower the rate. (*d*) That the complete removal of microbes from water, by filtration, is unattainable without frequent renewal of the best filtering materials.

'It is often urged that the bacteriological examination of water is of little practical importance, inasmuch as the microbes found are not necessarily prejudicial to health, and that the method of examination does not aim at the detection of harmful forms. A little more mature consideration, however, will show that the actual detection of harmful or pathogenic forms is a matter of very little importance; and if methods of water purification are successful in removing microbes in general, and more especially those which find a suitable home in natural waters, there can be no serious doubt that they will be equally successful in removing harmful forms, which are not specially adapted for life in water. Could it be, for instance, reasonably contested that a method of purification which is capable of removing the *Bacillus aquatilis* from water would be incapable of disposing of the *Bacillus anthracis*, when suspended in the same medium? The supposition is, on the face of it, absurd, and not a particle of experimental evidence can be adduced in its favour. It is, therefore, only rational to conclude that those methods of water purification, both natural and artificial, which succeed in most reducing the total number of microbes, will also succeed in most reducing the number of harmful forms should they be present’ (Frankland).

There are three methods by which microbes may be absolutely removed from water. These are by the agency of (*a*) electricity; (*b*) heat; (*c*) filtration through porous porcelain.

(*a*) *Electricity.*—The author[1] has shown that the electric current is capable of destroying the vitality of several microbes when growing in liquid media. For instance—

An E.M.F. of 2·16 volts destroys *Bacillus tuberculosis*,
,, ,, 2·26 ,, *Bacterium lactis*,
,, ,, 3·24 ,, *Bacterium aceti*,
,, ,, 3·3 ,, *Bacterium allii*,
,, ,, 2·72 ,, *Bacillus subtilis*;

and an E.M.F. of 180 volts readily destroys the

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[1] *Proceedings of Royal Society of Edinburgh*, vol. xv. p. 45; vol. xvii. p. 264; and *Researches on Micro-Organisms*, p. 177.

Protozoa contained in ordinary potable waters. Due to the author's investigations, Mr. R. Meade Bache[1] proposes to sterilise city waters by the agency of electricity. He says that 'after reading the results of Dr. Griffiths I gladly reverted to the intention with which I had set out in my experiments of being able to suggest means by which water supplied to cities could be sterilised for drinking purposes. The means at our command seem to me ample. It is true that we cannot electrolise successfully a large reservoir of water, for in that the electricity would be too diffused to be effective. It is true that, in pipes from which water is flowing into or out of the reservoir, its germs would not be subjected to attack for more than a second. It is true that the resistance that we should have to overcome in water would be large. But the electro-motive force (E.M.F.) of a few thousand volts (there are dynamos that generate ten thousand), thrown athwart a pipe of proper dimensions, would probably paralyse every bacterium in its path. . . . If, upon issuing from as well as upon entering a reservoir, the water were attacked in pipes from poles all but encircling them, with an electro-motive force of a few thousand volts, all germs must reach the denizens of cities supplied from such a source wholly innocuous, because they would be dead.'

Whether electricity is applicable or not for the sterilisation of water on a large scale, there is no doubt that it is a means by which microbes may be absolutely removed from water in the laboratory.

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[1] *Proceedings of American Philosophical Society*, vol. xxix. 1891), pp. 26-39.

(*b*) *Heat.*—Heat is a means of destroying microbes in water, but many microbes require a temperature above the boiling point before they are destroyed. Dr. Miquel[1] has shown that the number of microbes or colonies decreases gradually as the temperature of the water is raised. The water of the Seine, obtained from two different sources, gave the following results:—

Temperature of water (centigrade scale).	Average number of colonies per cc.	Temperature of water (centigrade scale).	Average number of colonies per cc.
At 20° . . .	464	At 22° . . .	848
,, 45° during 15 mins.	396	,, 43° during 15 mins.	640
,, 55° ,, ,, .	33	,, 50° ,, ,, .	132
,, 65° ,, ,, .	20·8	,, 60° ,, ,, .	40
,, 75° ,, ,, .	9·6	,, 70° ,, ,, .	27·2
,, 85° ,, ,, .	6·6	,, 80° ,, ,, .	26·4
,, 95° ,, ,, .	2·8	,, 90° ,, ,, .	14·4
,, 100° ,, ,, .	3·3	,, 100° ,, ,, .	5·2

Although some microbes are capable of withstanding the action of boiling water for 15 minutes, they are all destroyed when the temperature is raised to 110°–115° C. for the same space of time. MM. Rouart and Geneste-Herscher have devised an apparatus in which large quantities of water may be sterilised by the action of heat (see Miquel's book, *loc. cit.*, p. 188).

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[1] *Manuel Pratique d'Analyse Bactériologique des Eaux*, p. 182.

(*c*) *Filtration through porous porcelain.*—This is the last method for the absolute sterilisation of water. The Chamberland filter, which is universally used in bacteriological laboratories, is a device by means of which water is forced through porous porcelain. This filter may be attached to an ordinary water-tap if the pressure of the water-supply is sufficient to force the water through the porcelain; if not, a small force pump is required.

Certain bacteriologists classify waters according to the number of colonies revealed on cultivation. For instance, Miquel gives the following standard for the classification of waters:—

A water	excessively pure	yields	from	0 to 10	colonies	per cc.
,,	very pure	,,	,,	10 to 100	,,	,,
,,	pure	,,	,,	100 to 1,000	,,	,,
,,	ordinary (médiocre)	,,	,,	1,000 to 10,000	,,	,,
,,	impure	,,	,,	10,000 to 100,000	,,	,,
,,	very impure	,,	,,	100,000 or more	,,	,,

Such standards as this one are of little value, because a water which reveals only 10 to 100 colonies may be a worse water for drinking purposes than one which reveals 1000 colonies. The former may be contaminated with sewage, and, consequently, would form a suitable medium for the further development of microbes, whereas the latter may be free from sewage; therefore it would be the better water of the two. The fitness or otherwise of water for drinking purposes cannot be pronounced from the number of colonies obtained in a few quantitative determinations. 'It must never be forgotten that the sanitary examination of water is surrounded with such difficulties that it is only by bringing to bear on such particular case all the evidence that it is possible to obtain, and then interpreting this evidence by the light of an extended experience, that a sound judgment can be arrived at.'[1]

From what has been said in the last three chapters, it will be seen that we live in a world that is teeming with life. The air, the soil, the waters of ocean, river, and pond swarm with living microbes, each more or less perfectly adapted to the conditions of its existence. Many problems arise with regard to this world of living things; but suffice it to say that almost every drop of water which evaporates into the air carries with it germs, and there is no reason to suppose that the germs perish. On the contrary, there is much to lead us to believe that the germs have far greater powers of resisting high temperatures, desiccation, and other adverse conditions than the fully developed microbes. We may thus see how the air (and probably the soil) comes to be laden with germs which, should they fall into an appropriate infusion, or into water, may give rise to the teeming life which we know to be so soon developed in it.

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[1] For further information see Fabre-Domerque's *Manuel Pratique d'Analyse Micrographique des Eaux* (1890); Salazar and Newman's *Examen Químico y Bacteriológico de las Aquas Potables* (1890); Giglioli's *Fermenti e Microbi* (1887); Frank in *Zeitschrift für Anal. Chemie*, vol. xxx. p. 305; and Roux's *Précis d'Analyse Microbiologique des Eaux* (1891).

CHAPTER X

THE PTOMAÏNES AND SOLUBLE FERMENTS

THE advancement of organic chemistry has increased our knowledge of the alkaloids occurring in the vegetal kingdom—bodies which are of great importance both from a therapeutical and a toxicological aspect. Since the year 1872 a new source has been discovered of the natural origin of alkaloids, viz., from the animal kingdom, and the knowledge and investigation of these bodies have proved of great service in the study of both physiological and pathological chemical processes.

The ptomaïnes (πτῶμα = corpse) were first discovered in decomposing animal tissues, as their pseudonym of *cadaveric* alkaloids implies. Their presence in these dead tissues introduced a new factor in the *post-mortem* search for poisons in suspected cases of murder. This subject was brought into prominence by a murder trial in Rome, in which a man was accused of poisoning his master by administering delphinine. The accused was acquitted because the alkaloid obtained from the dead body differed in many of its reactions from those of delphinine; in other words, the poison extracted from the body was a ptomaïne produced by microbes after death. In 1882, G. H.

Lamson was accused of murdering his brother-in-law with aconitine, a vegetable alkaloid. The defence set up was that the alkaloid found in the dead body was one of the ptomaïnes produced after death. But it was conclusively proved by Drs. Dupré and Stevenson that the dead body contained aconitine which had been administered during life; consequently Lamson was executed[1] for murdering his brother-in-law. It will be seen from these remarks that the subject of ptomaïnes opens up an important point in all cases of poisoning where the poison is of an alkaloidal nature.

A more important result of the discovery of ptomaïnes has been the explanation of the cases of poisoning by decayed animal foods, such as sausages, fish,[2] 'tinned' and putrid meats, in which they have been found.

The ptomaïnes are produced during the process of putrefaction, etc., of animal substances. By the direct action of microbes, the albuminoid molecules are disintegrated with the formation of ptomaïnes among other products. From this remark, it will be seen that the ptomaïnes are not secreted or excreted by microbes, for they are the *residua* after microbian action. Not only have putrefactive microbes the power of giving rise to ptomaïnes, but certain pathogenic microbes yield ptomaïnes, or toxines, as Brieger calls them, when they are the products of microbian diseases.

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[1] At Wandsworth on April 28, 1882.

[2] See Dr. Griffiths' paper, 'The Poisoning of a Family by Mussels,' in *Chemical News*, vol. lxii. p. 17.

(*a*) *General properties of the Ptomaïnes.*—All the ptomaïnes (cadaveric) are soluble in alcoholic ether. Many of them dissolve in chloroform and amylic alcohol. The general reagents which precipitate the ptomaïnes are the following:—Myer's and Nessler's reagents, a solution of iodine in potassium iodide, the iodide of bismuth and potassium, and the phosphomolybdate of sodium. Mercuric chloride sometimes precipitates and sometimes does not precipitate the ptomaïnes, but it generally forms with them a double crystallisable chloride deposited from boiling water. Auric chloride often gives a yellow precipitate, soluble in water, or generally a very soluble aurochloride, which rapidly dissolves. Picric acid forms slightly soluble picrates. Tannin produces insoluble, as well as very slightly soluble tannates. Sulphuric acid diluted with a very small quantity of water produces a red-violet colour with the ptomaïnes. Hydrochloric acid gives a red-violet colour, which heat develops. Platinic chloride generally forms crystallisable compounds with the ptomaïnes. The majority of the ptomaïnes are crystallisable compounds, although a few are liquids. They are all, more or less, of a poisonous nature.

(*b*) *Extraction of the Ptomaïnes.*—The three most important methods for extracting ptomaïnes from putrefying and other material are those of Gautier, Brieger, and Luff. (1) *Gautier's method* consists in adding oxalic acid to the warm liquor of bacterial fermentation. By this means fatty or oily liquors are liberated, and float on the surface of the liquor. After separating the fats, the liquor is filtered. The filtrate is distilled, when pyrrol, skatol, phenol, indol, the volatile fatty acids, and a portion of the ammonia, are driven off. Gautier then adds lime (until alkaline) to the portion which has not been distilled, separates the precipitate which forms and which contains the greater portion of the fixed fatty acids, and he then distils the alkaline liquor to dryness *in vacuo,* taking care to condense the vapours in weak sulphuric acid. The bases are then distilled with ammonia. After the distillation is completed, the distillate is neutralised, then evaporated nearly to dryness, when ammonium sulphate deposits in the crystalline condition. This is separated and rejected. Concentrated alcohol is now added to the mother liquor, which dissolves the sulphates of the ptomaïnes. After evaporating off the alcohol, a small quantity of caustic soda solution is added. This solution is successively treated with ether, petroleum ether, and chloroform (*i.e.* three different extracts are obtained). As to the product remaining in the retort with the excess of lime which had served to separate the bases or ptomaïnes, it is treated with ether at 36° C., which dissolves the fixed bases. By the addition of a small quantity of acidulated water, the bases are separated from the ether, and are then easily precipitated by the addition of an alkali. (2) *Brieger's method* consists in boiling the putrefying material with water and then filtering. The filtrate is precipitated with plumbic acetate. This precipitate is filtered off a stream of sulphuretted hydrogen is passed through the filtrate, and the lead sulphide separated by filtration. This filtrate is evaporated to a thin syrup, and this is extracted with amylic alcohol. The amylic solution is treated with water, concentrated by evaporation, then acidulated with sulphuric acid, and washed several times with ether, which frees it from the oxy-aromatic acids. The aqueous-acid liquor is then concentrated to a quarter of its volume. After standing twenty-four hours, the precipitate which forms is dissolved in boiling water and decomposed by sulphuretted hydrogen. In concentrating the liquors, Brieger crystallises at once various mineral or organic salts which are rejected, then the dried residue is treated with absolute alcohol, which, after concentration, deposits the putrefactive bases or ptomaïnes in the crystalline condition. The various ptomaïnes are now separated by the difference of their solubility, or by fractional precipitation with platinic chloride, auric chloride, picric acid, etc.[1] (3) Luff's method is used for the extraction of ptomaïnes contained in abnormal urines. A considerable quantity of the urine is made alkaline by a solution of sodium carbonate, and then agitated with half its volume of ether. The ethereal solution (after standing) is filtered and agitated with a solution of tartaric acid. The tartaric acid combines with any ptomaïnes present, forming soluble tartrates, and the solution of tartrates forms the lower layer of the liquid mass. The tartaric acid solution (after being separated from the ether) is also made alkaline by the addition of sodium carbonate, and is once more agitated with half its volume of ether. The ethereal solution (after standing) is separated, and the ether allowed to evaporate spontaneously. The residue (after drying over sulphuric acid) is finally examined for ptomaïnes.

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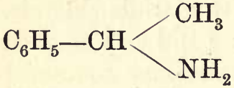
[1] See Brieger's *Ueber Ptomaine* 1885; *Weitere Untersuchungen über Ptomaine*, 1885; *Untersuchungen über Ptomaine*, 1886.

The ptomaïnes or animal alkaloids are divided into two groups — the non-oxygenous and the oxygenous ptomaïnes.

(A.) *The Non-oxygenous Ptomaïnes.*

Parvoline ($C_9H_{13}N$).—This base was isolated by Gautier and Etard, from mackerel and horse-flesh after bacterial putrefaction. It is an oily yellow base, with the odour of hawthorn.

Collidine ($C_8H_{11}N$) or isophenylethylamine  $C_6H_5{-}CH\begin{array}{l}\diagup CH_3\\ \diagdown NH_2\end{array}$ was discovered by Nencki in 1876, among the products of the bacterial putrefaction of gelatine and the pancreas of the ox. An isomeride of this ptomaïne was isolated by De Coninck from the muscular tissues of the cuttle-fish (*Sepia*); and it is probable that it has the constitutional formula of dihydropyridine. Collidine is a yellow fluid with an offensive odour; and it is soluble in water, alcohol, and ether.

Coridine ($C_{10}H_{15}N$).—This ptomaïne was extracted by Guareschi and Mosso from the bacterial putrefaction of fibrin. It is an oily fluid, having a slight odour of pyridine and conicine. It forms a crystalline platinochloride, an aurochloride, and a hydrochloride. De Coninck has extracted a base with the same formula from the muscular tissues of the cuttle-fish after bacterial putrefaction.

Hydrolutidine ($C_7H_{11}N$).—This base was extracted by Gautier and Mourgues from cod-liver oil. It is a colourless body, which is slightly oily and very alkaline. It is slightly soluble in water, and forms crystalline double salts with the chlorides of gold and platinum.

Hydrocollidine ($C_8H_{13}N$).—This ptomaïne was extracted by chloroform from the bacterial putrefaction of mackerel, horse-flesh, and other albuminous matters. It is a colourless liquid of the odour of syringa, and has a density of 1·029 at 0° C. This base forms crystalline double salts with the chlorides of hydrogen, gold, and platinum. Hydrocollidine is very poisonous; in fact, Gautier says of this ptomaïne: 'Elle détermine du tremblement, des convulsions tétaniques, l'animal meurt avec le cœur en diastole gorgé de sang.'

Hydrocoridine ($C_{10}H_{17}N$).—This base was extracted by the author[1] from pure cultivations of *Bacterium allii* on nutrient agar-agar. It is a white solid, soluble in warm water, alcohol, ether, and chloroform. It crystallises from water in microscopic needles belonging to the prismatic system. These crystals are extremely deliquescent, and have the odour of hawthorn, especially when heated. This ptomaïne forms crystalline double salts with the chlorides of platinum and gold.

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[1] See Griffiths' papers in *Comptes Rendus de l'Académie des Sciences*, vol. cx. p. 418; *Centralblatt für Bakteriologie und Parasitenkunde*, Bd. 7, p. 808; *Chemical News*, vol. xli. p. 145; and Gautier's *Chimie Biologique* (1892), p. 268.

It appears that the ptomaïnes just described belong to, or are derivatives of, the pyridine series of organic bases.

Neuridine ($C_5H_{14}N_2$).—This ptomaïne was discovered by Professor Brieger as a constant product of the bacterial putrefaction of albuminous substances. Its hydrochloride, platinochloride, and aurochloride have been obtained in the crystalline condition; but the free base is so unstable that it has never been obtained pure. A solution of sodium hydroxide decomposes neuridine hydrochloride into dimethylamine and trimethylamine.

Saprine ($C_5H_{14}N_2$).—This base is isomeric with the preceding, but differs from it in the solubilities of its salts, and probably also in chemical constitution. It was discovered by Brieger in 1883.

Cadaverine ($C_5H_{14}N_2$).—Brieger isolated a third isomeride from albuminous substances subjected to prolonged putrefaction. This base is also readily formed in cultivations of Finkler's spirillum. Dr. Ladenburg[1] proved that this ptomaïne has all the chemical and physical properties of pentamethylenediamine. It is a non-poisonous liquid with an alkaline reaction. This base boils at 115° C., and it has the odour of conicine.

Putrescine ($C_4H_{12}N_2$) is usually found accompanying cadaverine, but makes its appearance rather later. It is a volatile liquid, with a spermatic odour, and boils at 135° C. Baumann and Udranszky proved that this ptomaïne has the constitutional formula of tetramethylenediamine, $NH_2(CH_2)_4NH_2$. Both cadaverine and putrescine have been isolated from the fæces and urine in cases of cystinuria.[2]

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[1] *Berichte der deutschen chemischen Gesellschaft*, vol. xix. p. 2586.

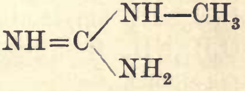
Mydaleine.—This base was isolated (along with cadaverine and putrescine) by Brieger during the putrefaction of albuminous substances. It is a poisonous ptomaïne which causes paralysis and death. Mydaleine is believed to be a diamine, but it has not been thoroughly examined.

Brieger has also isolated two ptomaïnes from pure cultivations of the tetanus bacillus, which are probably diamines. One is called spasmotoxine, and produces tonic and clonic convulsions, while the other (which has not been named) causes tetanus, accompanied with a flow of saliva and tears.

Tyrotoxicon ($C_6H_5N_2$).—In 1886, Vaughan isolated this ptomaïne from cheese, milk, and ice-cream which had undergone putrefaction. Tyrotoxicon produces nausea, diarrhœa, and acute poisoning, and is said to be identical with diazobenzene.

Phlogosin (formula unknown) is a ptomaïne which was obtained by Leber in 1888 from pure cultivations of *Staphylococcus aureus.* It is probably a diamine.

Methylguanidine $C_2H_7N_3$ or



$$NH{=}C\begin{cases} NH{-}CH_3 \\ NH_2 \end{cases}$$

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[1] See also Dr. Lauder Brunton's *Disorders of Digestion*, p. 281.

is a very poisonous base, and it is produced when Finkler's spirillum is allowed to live upon sterilised beef along with putrefactive microbes. This substance is believed to be the cause of cholera nostras.

Spermine (C_2H_5N) or dimethylenimide $(CH_2)_2NH$ occurs in the seminal fluid of animals, and according to Schreiner it is produced in cultivations of the tubercle bacillus, but the author could not detect the least trace of this base in pure cultivations of *Bacillus tuberculosis.*

Ethylenediamine ($C_2H_8N_2$).—In 1885, Brieger extracted this ptomaïne from cod-fish after bacterial putrefaction. It forms crystallisable double salts with the chlorides of hydrogen and platinum.

Tetanotoxin ($C_5H_{11}N$).—This ptomaïne was extracted by Brieger from pure cultivations of the tetanus bacillus. It is a colourless liquid which boils at 100° C., and possesses a disagreeable odour. When it is injected into animals it produces tremor and paralysis, followed by violent convulsions.

Methylamines.—Methylamine CH_3NH_2, dimethylamine $(CH_3)_2NH$, and trimethylamine $(CH_3)_3N$, have been extracted from the tissues of various animals. Triethylamine $(C_2H_5)_3N$ and propylamine $C_3H_7NH_2$ have been obtained from putrified animal substances. While Gautier and Mourgues extracted ptomaïnes, having the composition of butylamine $C_4H_9NH_2$, amylamine $C_5H_{11}NH_2$, and hexylamine $C_6H_{13}NH_2$ from cod-liver oil. These bases are poisonous.

Scombrine ($C_{17}H_{38}N_4$).—This base was discovered by Gautier and Etard[1] in certain extracts of mackerel which had undergone bacterial putrefaction. It has the odour of syringa, and is decomposed at 100° C. Scombrine forms a crystallisable platinochloride which is soluble in water.

Morrhuine ($C_{19}H_{27}N_3$).—This ptomaïne was isolated by Gautier and Mourgues from cod-liver oil. It is a yellowish liquid with the odour of syringa, and is very alkaline and caustic.

Aselline ($C_{25}H_{32}N_4$).—This base was also isolated by the same authorities from cod-liver oil. It is an inodorous solid, which is soluble in ether and alcohol, but insoluble in water. In large doses it is poisonous.

(B.) *The Oxygenous Ptomaïnes.*

Propylglycocyamine ($C_6H_{13}N_3O_2$.)—This ptomaïne was extracted by the author[2] from the urine in a case of parotitis or mumps, where the kidneys were involved. It crystallises in white prismatic needles, which are soluble in water, ether, and chloroform. This base has a neutral reaction, a slightly bitter taste, and forms a yellow crystalline platinochloride, a pale yellow aurochloride, and a white crystalline hydrochloride. When boiled with oxidising agents it yields creatine (methylglycocyamine) and finally methylguanidine and oxalic acid. This ptomaïne has the constitutional formula of propylglycocyamine

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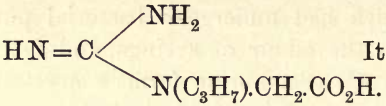
[1] See Gautier's *Chimie Biologique* (1892), p. 268.

[2] *Comptes Rendus*, tome cxiii. p. 656; *Chemical News*, vol. lxi. p. 87; and *Bulletin de la Société Chimique de Paris*, 3e série, tome iv. p. 333.



$$HN=C\begin{array}{l}\diagup NH_2\\ \diagdown N(C_3H_7).CH_2.CO_2H.\end{array}$$

It is poisonous and when administered to a cat it produced nervous excitement, cessation of the salivary flow, convulsions, and death. This base is not found in normal urines, it is therefore produced within the system during the course of the disease, which is highly infectious.

Neurine ($C_5H_{13}NO$).—This ptomaïne is a constant product of cadaveric putrefaction. It is a syrupy base, soluble in water, and has a strong alkaline reaction. It forms a crystallisable platinochloride, besides other double salts. It is a poisonous base: 0·01 gram. of neurine kills a cat, and 0·04 gram. kills a rabbit. Brieger states that this ptomaïne has the same formula as trimethylvinylammonium hydroxide $(CH_3)_3(C_2H_3)N.OH$.

Choline ($C_5H_{15}NO_2$).—Like neurine, this ptomaïne is a constant product of cadaveric putrefaction. It is a syrupy base, soluble in alcohol and ether, and has a strong alkaline reaction. It forms double salts with the chlorides of hydrogen, gold, and platinum; and it also forms compounds with carbonic and sulphuric acids. When heated, choline is decomposed into glycol and trimethylamine. Choline in small doses produces pyrexia: in larger doses it produces paralysis due to poisoning of the motor end-plates. This ptomaïne has the same constitutional formula as trimethyloxyethyleneammonium hydroxide $(CH_3)_3(C_2H_4—OH)N.OH$.

Muscarine ($C_5H_3NO_2$).—Brieger isolated this ptomaïne from putrid fish. It also occurs in the poisonous mushroom (*Agaricus muscarius*). Muscarine is a crystalline deliquescent substance, which acts on the muscular tissues.

Gadinine ($C_7H_{16}NO_2$) was obtained by Brieger, along with muscarine, from putrefying cod-fish. It forms double salts with the chlorides of hydrogen, gold, and platinum.

Mytilotoxine ($C_7H_{15}NO_2$) was isolated by Brieger from decomposing mussel, and it is the active agent in mussel-poisoning.

Typhotoxine ($C_7H_{17}NO_2$).—This ptomaïne was extracted by Brieger from pure cultivations of the typhoid bacillus. It has an alkaline reaction, and forms crystallisable salts with phosphotungstic acid and the chlorides of hydrogen and gold. It is believed to be the chemical poison in typhoid fever.

In 1886 Brieger obtained an isomeride of this base from flesh which had undergone bacterial putrefaction. Although this substance has the same empirical formula as typhotoxine, they are entirely different ptomaïnes.

Scarlatinine ($C_5H_{12}NO_4$).—This ptomaïne was extracted by the author[1] from the urine of patients suffering from scarlet fever, as well as from pure cultivations of *Micrococcus scarlatinæ*. It is a white crystalline body, which is soluble in water, and has a faint alkaline reaction. It forms double salts with the chlorides of hydrogen and gold, and it is precipitated by phosphomolybdic, picric, and phosphotungstic acids.

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[1] Griffiths in *Comptes Rendus de l'Académie des Sciences*. vol. cxiii. p. 656; *Proc. R.S.E.*, vol. xix. p. 97.

Tetanine ($C_{13}H_{22}N_2O_4$).—Brieger succeeded in isolating this ptomaïne from pure cultivations of the tetanus bacillus. This base produces tetanic convulsions and death. Its hydrochloride is very deliquescent. In 1888 Brieger[1] obtained another oxygenous ptomaïne from pure cultivations of the tetanus bacillus. It is represented by the formula $C_6H_{13}NO_2$, and is non-poisonous.

Diphtherine ($C_{14}H_{17}N_2O_6$).—This base was extracted by the author[2] from the urine of patients suffering from diphtheria, as well as from pure cultivations of *Bacillus diphtheriæ* (bacillus No. 2 of Klebs and Löffler). It is a white crystalline base, and it forms double salts with the chlorides of hydrogen and gold. It is precipitated by tannic, picric, and phosphomolybdic acids.

Unknown Base ($C_5H_{11}NO$).—This ptomaïne was extracted by E. and H. Salkowski from putrid fibrin. It forms double salts with the chlorides of hydrogen and platinum. Gabriel and Aschan[3] have recently proved that this ptomaïne is δ-amidovaleric acid.

Unknown Base ($C_{14}H_{20}N_2O_4$).—This base, which is believed to be an amido-acid, was extracted by Guareschi[4] from putrid fibrin. It occurs as beautiful shining plates, which melt at about 250°C. This ptomaïne is soluble in water, ether, and chloroform.

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[1] *Virchow's Archiv*, Bd. 112, p. 550; Bd. 115, p. 490.

[2] *Comptes Rendus*, vol. cxiii. p. 656.

[3] *Berichte der deutschen chemischen Gesellschaft*, vol. xxiv. p. 1364.

[4] *Annali di Chimica e di Farmocologia*, vol. lxxxvii. p. 237.

Mydine ($C_8H_{11}NO$). — This ptomaïne was extracted by Brieger from human corpses and pure cultivations of the typhoid bacillus. It is non-poisonous, and its picrate melts at 195° C.

Betaine ($C_5H_{11}NO_2$) or trimethylglycocine was first isolated from urine by Liebreich in 1869. It is related to neurine and choline. In 1885, Brieger extracted the same base from poisonous and non-poisonous mussels. Betaine is a non-poisonous ptomaïne.

Pyocyanin ($C_{14}H_{14}NO_2$).—This is the greenish pigment produced by *Bacillus pyocyaneus.* It is soluble in water, alcohol, and chloroform. According to Ledderhose, this compound appears to be a derivative of anthracene.

Unknown Bases ($C_7H_{18}N_2O_6$ and $C_5H_{12}N_2O_4$).—Dr. G. Pouchet extracted both of these bases from putrid animal substances. They are very poisonous, and give rise to crystalline hydrochlorides and platinochlorides.

Sucholotoxine.—This is a ptomaïne extracted from pure cultivations of the microbe of hog cholera. It forms double salts with the chlorides of platinum and hydrogen. Von Schweinitz[1] states that this base is very poisonous.

Suplagatoxine. — This was extracted by Von Schweinitz[2] from pure cultivations of the microbe of swine plague. It is also poisonous. The chemical formulæ of Von Schweinitz's ptomaïnes have not been ascertained.

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[1] *Journ. Amer. Chem. Soc.*, 1891.

[2] *Ibid.*

Various bases, of unknown composition, have been extracted from urine, fæces, and tissues in certain infectious and contagious diseases; and in addition to these there is another class of animal alkaloids which have been termed leucomaïnes by Gautier. According to Gautier the leucomaïnes are excretory products (like urea, carbonic acid, etc.) formed by 'vital physiological processes' from albuminous substances, consequently they must be eliminated from, or destroyed in, the system, or disease will be the result of their poisonous action. We resist, therefore, incessant auto-infection by two distinct mechanisms; the elimination of the leucomaïnes by means of the excretory organs, and by the destruction of the leucomaïnes by means of the oxygen contained in the blood. 'Some of these leucomaïnes are exceedingly poisonous, and when retained may give rise to very serious toxic symptoms. Brieger and others, however, deny that any such bodies are formed, or at any rate have yet been found in the tissues of the living body, or that they owe their existence to the tissues. They consider that they are simply absorbed from the intestinal canal where they are formed by bacteria' (Woodhead).[1]

It should always be borne in mind that 'the discovery of ptomaïnes is complemental, not antagonistic, to the germ theory.'

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[1] A full description of the leucomaïnes is given in the author's book, *Researches on Micro-Organisms*, pp. 121-134.

In addition to the ptomaïnes, there are the enzymes and albumoses, which are chemical principles, excreted by microbes and allied fungi, or the products of the activity of other living cells, *e.g.* those of the glands of the stomach, pancreas, etc. Such soluble ferments or enzymes as pepsin, ptyalin, trypsin, diastase, invertin, and emulsin are well known to physiologists and chemists. It is not, however, these bodies which we intend to describe, but the albumoses produced by living microbes. In small doses these albumoses are protective; and they appear to be the protective principles in most vaccines.

'The albumoses produced by microbes resemble those formed during normal digestion in being poisonous when injected directly into the circulation, although they may not be so greatly absorbed from the intestinal canal. One of the most remarkable discoveries in regard to albuminous bodies is the fact that some of them which are perfectly innocuous, and, indeed, probably advantageous to the organism in their own place, become most deadly poisons when they get out of it. Thus the thyroid and thymus glands, which are perfectly harmless and probably useful, were found by Wooldridge, when broken up in water, to yield a proteid which instantaneously coagulated the blood if injected into a vein, so that the animal died as if struck by lightning; while Schmidt-Mühlheim, under Ludwig's direction, found that peptones had an exactly opposite effect, and prevented coagulation altogether.'[1]

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[1] Dr. Lauder Brunton in *Nature*, vol. xliv. (1891), p. 330.

Dr. Bitter, in 1887, 'furnished rigorous proof that microbes produce albumoses separable from the organisms which form them. He managed to kill the microbes by sterilisation at 60°C. without materially destroying their products, and in this way demonstrated that two microbes, when grown on gelatine, produced albumoses which were able, apart from the microbes, to liquefy gelatine and peptonise albumin.' In the same year Löffler, after separating the microbes by means of a Chamberland filter, obtained an albumose from pure cultivations of *Bacillus diphtheriæ*. This albumose is precipitated by alcohol, and is soluble in water. Roux, Yersin, Brieger, and Fränkel have obtained a similar substance from cultivations of the same microbe. This albumose produces all the characteristic symptoms of diphtheria ; therefore, *B. diphtheriæ*, which excretes this poisonous albumose, or toxalbumin, as Brieger calls it, is really the cause of the disease.

Hankin[1] has extracted an albumose from cultivations of anthrax bacilli. It is precipitated by alcohol, and is soluble in water. Martin[2] obtained two albumoses from pure cultivations of the same microbe. These albumoses are strongly alkaline; but they are not so toxic as the ptomaïne which *B. anthracis* is said to produce.

Among the microbes which excrete albumoses are the following:—

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[1] *Proc. Roy. Soc.*, May 22, 1890.

[2] *Ibid.*

Bacillus diphtheriæ.
Bacillus anthracis.
Bacillus tuberculosis.
Spirillum choleræ Asiaticæ.
Bacillus of hog cholera.
Spirillum tyrogenum.
Bacillus of swine plague.
Bacillus typhosus.
Bacillus of tetanus.
Staphylococcus aureus.
Spirillum Finkleri.
Bacillus ureæ.
Bacillus butyricus.
Bacillus malariæ (?).

Drs. Brunton and Macfadyen[1] have proved that the albumoses, excreted by certain microbes, have the power of liquefying gelatine; and there is every reason to believe that the liquefaction of gelatine, during the cultivation of microbes, is due to the action of albumoses.

Very little is known of the composition of the albumoses; but their reactions with certain reagents (Millon's fluid, magnesium sulphate, copper sulphate and potash, etc.) prove that they are derived from proteids. They are neither albumins nor globulins; in other words, they belong to the albumose group of bodies.

Two albumoses, sucholoalbumin and suplagoalbumin, which Von Schweinitz[2] extracted from pure cultivations of the microbes of hog cholera and swine plague respectively, are white, pulverulent substances, soluble with difficulty in water, and precipitated from this solution by absolute alcohol. They can be obtained in crystalline plates by drying over sulphuric acid *in vacuo*.

In addition to the ptomaïnes and albumoses, other substances are formed by microbes. Among these are the various coloured pigments; but the chemistry of the microbian pigments is a subject which has been very little investigated. They are undoubtedly products formed from the decomposition of albuminoids by the agency of microbes.[1]

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[1] *Proc. Roy. Soc.*, vol. xlvi. (1889), p. 542.

[2] *Journ. Amer. Chem. Soc.*, 1891.

In concluding the chapter, it may be stated that the substances which microbes produce put a stop to their activity; thus the alcohol produced by yeast, the phenol, cresol, etc., produced by putrefactive microbes, are themselves germicides, which ultimately kill the organisms that produce them.

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[1] Concerning the composition of the red pigment produced by *M. prodigiosus*, see the author's paper in *Comptes Rendus*, vol. cxv. p. 321. The green pigment—pyocyanin—has already been described (p. 319). The author has described ptomaïnes in glanders, pneumonia, and puerperal fever; and also one produced by *M. tetragonus* (see *Comptes Rendus*, vols. cxiv. and cxv.).

CHAPTER XI

GERMICIDES AND ANTISEPTICS

THE substances which destroy the vitality of microbes are called germicides or disinfectants; while those which simply retard or hinder the growth of microbes are generally spoken of as antiseptics. It must be borne in mind that this is only a conventional classification or division, for a germicide may become an antiseptic by simply reducing its strength; and, conversely, an antiseptic (as a rule) may become a germicide by increasing its strength.

Among the more common salts Mr. C. T. Kingzett[1] has proved that the chlorides, nitrates, and sulphates of the alkalis exhibit but slight antiseptic and germicidal effects, and those of the alkaline earths are not much better. The same salts of manganese, zinc, tin, iron, lead, and aluminium are all of more or less pronounced value. As a rule the chlorides are to be preferred. The same salts of copper and mercury are comparatively most effective; the nitrate of mercury is, however, not so reliable as the chloride, which is, according to

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[1] *Journ. Soc. Chem. Industry*, vols. vi. and vii.

Kingzett, the most active antiseptic and germicide among these classes of substances.

Edington[1] has shown that mercuric chloride dissolved in water (rendered acid) in the proportion of 1 part in 1000 destroys the spores of *Bacillus anthracis* in fifteen minutes, for the spores after this treatment and subsequent washing in sterilised water refused to grow on nutrient agar-agar. Mercuric chloride also destroys *Bacterium allii, Micrococcus tetragonus, M. prodigiosus, M. violaceus, Sarcina lutea, Bacillus subtilis,* and other microbes.[2] Perhaps a more powerful germicide than mercuric chloride is mercuric iodide; and Woodhead[3] has used a solution containing '1 gramme of mercuric iodide with a slight excess of potassium iodide in 1000 cc. of distilled water.'

Chlorine gas and the vapours of bromine and iodine are powerful germicides, readily destroying most microbes. According to the author's[4] investigations, the germicidal power of the three halogen elements is inversely as their atomic weights (Cl = 35·5; Br = 80; I = 127), *i.e.* chlorine is the most powerful germicide, then bromine, and finally iodine. In fact, the germicidal power of these elements coincides with their chemical affinities; but this remark does not apply to the salts containing these elements. Iodine,[5] potassium iodide, sodium iodide, ethyl iodide, potassium iodate,[1] bromine, ethyl bromide, chlorine, ferric chloride, and sodium fluosilicate[2] ('salufer') destroy many microbes, including *Bacillus tuberculosis, Sarcina lutea, Bacterium allii, B. œdematis maligni,* and *B. subtilis.*

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[1] *British Medical Journal*, 1889.

[2] Griffiths' *Researches on Micro-Organisms*, p. 204.

[3] *Proceedings of Royal Society of Edinburgh*, vol. xv. p. 246.

[4] *Loc. cit.*, p. 182.

[5] Griffiths in *Proc. Roy. Soc. Edinburgh*, vol. xv. p. 37.

Many of the derivatives of benzene and its homologues are powerful germicides. Among these compounds may be mentioned the following: benzoic acid, sodium benzoate, sodium benzenesulphinate, salicylic acid, sodium salicylate, carbolic acid, sodium carbolate, etc.; and the late Dr. T. Carnelley[3] proved that 'the para-compounds (of benzene) are usually more powerfully antiseptic than the corresponding ortho- and meta-compounds.'[4] For instance, it has been shown that of the three sodium nitrobenzoates, it required 101·6 grammes of the ortho-compound, 12·1 grammes of the meta-compound, and 7·7 grammes of the para-compound respectively to sterilise 1 litre of nutrient gelatine. There are, however, a few exceptions to this rule; among these are the three sodium hydroxybenzoates: it required 11·6 grammes of the ortho-compound (sodium salicylate), 67·2 grammes of the meta-compound, and 162·1 grammes of the para-compound respectively to sterilise 1 litre of nutrient gelatine.

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[1] Griffiths in *Proc. Roy. Soc. Edinburgh*, vol. xvii. p. 257.

[2] Thomson in *Chemical News*, vol. lvi. p. 132.

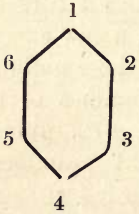
[3] *Journal of Chemical Society*, 1890, p. 636.

[4] It may be stated that a derivative of benzene with a certain empirical formula may exist in three isomeric modifications. Although these isomerides have the same empirical formula, their constitutional formulæ, and consequently their properties, are entirely different. This difference depends upon the relative positions of the elements or groups of elements introduced into the molecules. If we represent the orientation of the side-

The author[1] has shown that a saturated solution of salicylic acid destroys *Sarcina lutea, M. prodigiosus, Bacillus tuberculosis, Bacterium allii, M. tetragonus, Bacterium lactis, Bacterium aceti, M. aurantiacus, Bacillus subtilis, Leptothrix buccalis, M. ureæ, Bacillus butyricus,* etc. It should be borne in mind that salicylic acid is a more powerful germicide than sodium salicylate; and that the natural salicylic acid is a more powerful germicide than the artificial variety.

Koch has proved that a 3 per cent. solution of carbolic acid completely destroyed the spores of *Bacillus anthracis* in seven days, while a 5 per cent. solution destroyed them in two days. A 1 per cent.

chains in benzene derivatives by numerals, the terms ortho-, meta-, and para-compounds are readily understood :—



The term *ortho-* is always applied to the positions 2 and 6 in relation to 1.

The term *meta-* is always applied to the positions 3 and 5 in relation to 1.

The term *para-* is always applied to the position 4 in relation to 1.

[1] *Proc. Roy. Soc., Edinburgh,* vol. xiii. p. 527; vol. xiv. p. 97; vol. xv. p. 37; vol. xvii. p. 257; and *Researches on Micro-Organisms,* p. 223 *seq.*

solution easily destroyed the sporeless bacilli, but in a ·5 per cent. solution they were not destroyed.

As an antiseptic agent carbolic acid, in the shape of dressings and lotions, and as a spray in surgical operations, is of the greatest value; but, according to Jalan de la Croix,[1] its germicidal properties are inferior to those of salicylic acid.

Among the oxidising germicides and antiseptics are the following:—Hydrogen dioxide, ozone, 'sanitas oil,' 'sanitas fluid,' potassium permanganate (Condy's fluid), and turpentine oil. All these substances have germicidal and antiseptic properties, which are due (directly or indirectly) to the liberation of nascent oxygen.

The author has shown that when silk threads, impregnated with tubercle bacilli and the bacilli of hay fever, were placed in a mixture containing 5 cc. of 'sanitas oil' and 100 cc. of water for seven days, the microbes were completely destroyed by this powerful oxidising agent.

Mr. C. T. Kingzett, F.C.S.,[2] has performed a large number of experiments with 'sanitas oil' and 'sanitas fluid,' and his experiments prove the high value of these preparations as germicidal and antiseptic agents.

In addition to the above-mentioned germicides and antiseptics there are many others, among these being the following:—Sulphurous anhydride, alkaline sulphides and hyposulphites, hydrogen sulphide quinine, α- and β- naphthol, arsenious acid, sodium arsenite, potassium arsenite, arsenic acid, alcohols, boric acid, certain essential oils, etc. Heat, electricity, and certain gases have also the power of destroying microbes.

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[1] *Archiv für Experim. Pathol.*, vol. xiii.

[2] *Nature's Hygiene* (3d ed.), pp. 319-351.

As antisepsis and disinfection play such important parts in medicine, surgery, and sanitation, it is desirable that greater attention should be paid to the investigation of the action of various chemicals, etc., on microbes than has hitherto been the case.

CONCLUDING REMARKS.

We have seen that microbes are omnipresent, being so light in weight they are readily carried over thousands and thousands of miles by air currents without losing their vitality. This is not surprising when we bear in mind that Rome has been showered with the sands of Sahara, France with South American diatoms, and that the volcanic dust from Cotopaxi fell thousands of miles away from the seat of the eruption. If sands, diatoms, and volcanic dust are capable of being carried enormous distances, it is hardly irrational to suppose that microbes may travel from planet to planet, especially the anaërobic forms, and even those which are aërobic are capable of being desiccated without losing their vitality.

Then again, from these first living germs, in which the peculiarities of the animal and vegetal kingdoms are hardly yet separated, the laws of development, the struggle for existence, natural increase, geographical distribution, and many other known and unknown forces might have produced the different forms of the animal and plant world, which inhabited the earth in the past as they do in the present. We know that in the maintenance of such views we stray far from the boundaries of biological science; but we find the biologist, always remaining conscious of the limitations of his knowledge, admitting his ignorance with resignation, foiled in his experiments and observations, not always resisting the longing of Faust, 'Zu schauen alle Wirkungskraft und Samen,' but gladly giving himself up to the allurement of filling with some fantasy that blank in which modern investigation has failed.

APPENDIX

I. THE MEASLES BACILLUS

DRS. CANON and PIELICKE,[1] of Berlin, have recently discovered the true microbe of measles. They have examined the blood of fourteen patients suffering from measles, and in all cases found the same microbe. Microscopic slides of the blood were stained with eosin-methylene-blue; the bacilli being stained blue. They differ in size, being sometimes as long as the radius of red blood corpuscle, sometimes as large again, and sometimes smaller; similar bacilli were also found in the expectorations, and in the various secretions of the patients. They grow in artificial media.

II. MICROCOCCUS TETRAGENUS CONCENTRICUS.

Dr. Schenk,[2] of Vienna, has discovered in the stomach of patients suffering from intestinal catarrh a new microbe, which he calls *M. tetragenus concentricus.* It is not known whether this microbe is identical with that found in the bodies of those who have recently died from stomachic influenza.

[1] *Deutsche Medicinische Wochenschrift*, 1892.

[2] *Wiener Allgemeine Medizinische Zeitung*, Feb. 1892

UNIVERSITY OF CALIFORNIA

III. THE INFLUENZA BACILLUS.

Drs. Pfeiffer, Kitasato, and Canon have (independently of one another) discovered the influenza microbe. It has been found in the saliva and the bronchial discharges characteristic of influenza. It exists in the form of small rodlets, strung together in threads. It grows in agar-agar and sugar, or in agar-agar and glycerine. In the saliva of influenza patients, the bacilli are found in large numbers; they may penetrate from the pus cells into the tissue of the lungs, and even pass as far as the surface of the pleura. This fact explains the rapidity and fatality of lung complications in influenza. The same bacillus has also been found in the blood of patients suffering from the disease.

The knowledge that a bacillus residing in the saliva causes influenza will not cure the epidemic; but the prompt and practical application of this knowledge by complete disinfection of all bronchial and nasal secretions, and the isolation of influenza patients will arrest the plague. It also indicates the reasonableness of what is known as the carbolic acid treatment of influenza, which has been practised with considerable success, especially in the early stages of the disease.

IV. BACILLUS PLUVIATILIS.

The author[1] discovered this microbe in rain-water, contained in a barrel, and exposed to the air during certain mild weeks in the winter of 1890. At this period of the year, the majority of the other microbes in the water were in an inactive condition, consequently the struggle for existence was reduced to a minimum. It is probable that this rare microbe is an aërial form, but the author has not found it in the atmosphere.

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[1] Dr. A. B. Griffiths, *Bulletin de la Société Chimique de Paris*, 1892, 3e série, tome vii. p. 332.

B. pluviatilis grows well on gelatine plates, and in four days forms a small yellow colony, with liquefaction of the gelatine. The growth of this microbe in gelatine-tubes is also characteristic, and in from thirty-six to forty-eight hours after inoculation, it forms a thin yellowish band with a number of small lateral filaments. On the surface of the gelatine, there is developed a brilliant yellow colony. In bouillon at 30° C. this microbe forms a yellowish pellicle on the surface, and ultimately a flocculent deposit of the same colour settles at the bottom of the tube. It grows very rapidly on potatoes, giving rise to an orange growth which extends over almost the whole surface of the potato.

B. pluviatilis occurs in pairs and threads; and individual bacilli vary in length from 2 to 4 μ, and in breadth from 0·6 to 0·8 μ. This microbe, which stains well with the aniline colours, does not produce spores. It forms, in peptonise gelatine, a white crystalline ptomaïne having the formula $C_9H_{21}N_2O_5$.

Neither the microbe nor ptomaïne possesses any pathogenic properties.

V. THE CANCER BACILLUS.

Scheuerlein has cultivated a special microbe from cancerous tissues, which he considers as the veritable agent in producing cancer. This microbe grows well on solidified blood serum at 39° C.; and after three days' incubation, the whole surface of the medium is covered with a colourless pellicle. After many days or weeks, a brownish-yellow colour is developed, and ultimately the pellicle assumes the appearance of small liquid drops.

This microbe also grows on agar-agar, nutrient gelatine, potatoes, and in bouillon. It measures from 1·5 to 2·5 μ in length and 0·5 μ in breadth, and forms spores. According to Scheuerlein,[1] the same microbe is present in most malignant growths (especially sarcoma and carcinoma), and when pure cultures of it were injected into the mammary glands of bitches, they gave rise to small soft tumours. On no occasion has Scheuerlein failed to produce these tumours, consequently he believes that he has discovered the true factor in the ætiology of cancer. It may be remarked, *en passant*, that Domingos Freire[2] has confirmed the results of Scheuerlein; but there is no doubt that they still require further confirmation.

VI. THE MICROBE OF WHOOPING COUGH.

Afanassieff[3] has found a bacillus in the pearly phlegm of persons suffering from whooping cough. This microbe, which measures from 0·6 to 2·2 μ in length, is readily cultivated on gelatine plates, where it forms small round or oval colonies of a brownish colour. It is in shape what the French call bâtonnet;[4] and Afanassieff, who injected pure cultures of this microbe into the trachea of dogs and cats, has produced the typical symptoms of whooping cough in these animals.

---

[1] *Deutsche Medicinische Wochenschrift*, 1887, p. 1033.

[2] *Société de Médecine Interne de Berlin*, 1887.

[3] *St. Petersburger Medicinische Wochenschrift*, 1887.

[4] Pointed at both ends, like a tip-cat.

VII. PURE FERMENTATIONS AND MICROBES.

Frankland and Frew[1] have recently studied the action of *Bacillus ethacetosuccinicus* (which they discovered) on dulcitol and mannitol. The decomposition of these substances may be regarded as involving two independent reactions, viz. :—

$$\begin{aligned}(a)\ & C_6H_{14}O_6 = 2\,C_2H_6O + CO_2 + CH_2O_2 \\ (b)\ & C_6H_{14}O_6 = C_4H_6O_4 + C_2H_4O_2 + 2\,H_2;\end{aligned}$$

but from the proportion which the acetic acid bears to the alcohol, it appears that two molecules are resolved, in accordance with equations *a*, for every one that is decomposed according to *b*. Or, in other words, the decomposition of either dulcitol or mannitol by this microbe is represented by the following equation :—

$$3\,C_6H_{14}O_6 = 4\,C_2H_6O + 2\,CO_2 + 2\,CH_2O_2 + C_4H_6O_4 + C_2H_4O_2 + 2\,H_2.$$

The microbe which produces this change measures from 1·7 to 2·5 μ in length and from 0·5 to 1 μ in breadth. It occurs generally in pairs, and does not produce spores.

Frankland and Lumsden[2] have studied the decomposition of mannitol and dextrose by *Bacillus ethaceticus.* The products of the fermentation of both these compounds consists of ethyl alcohol, acetic acid, hydrogen, carbon dioxide, and traces of succinic acid. When the fermentations are conducted in a closed space, there is invariably also a considerable quantity of formic acid produced, whilst in fermentations in an open space (flasks plugged with cotton wool), formic acid, except in traces, is a most exceptional product. The proportions in which the several products are obtained from mannitol is approximately represented by the equation:

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[1] *Journal of Chemical Society*, 1892, pp. 254-277.

[2] *Proceedings of Chemical Society*, 1892, p. 70.

$$3\,C_6H_{14}O_6 + H_2O = C_2H_4O_2 + 5\,C_2H_6O + 2\,CH_2O_2 + CO_2,$$

whilst in the case of the dextrose, the products occur in the proportions:—

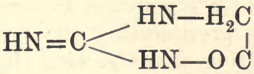
$$2{\cdot}5\,C_2H_6O \;:\; 1{\cdot}5\,C_2H_4O_2 \;:\; 3\,CH_2O_2 \;:\; CO_2.$$

There is a close resemblance between these fermentations by *B. ethaceticus* and those produced through the agency of Friedländer's micrococcus, which renders it probable that this ethacetic decomposition is a very general and typical form of fermentative change (Frankland).

VIII. PTOMAÏNES.

The author[1] has extracted the following ptomaïnes from urine in cases of measles, whooping-cough, and erysipelas:—

(a) From Measles.—The ptomaïne produced during the course of this disease is a white substance which crystallises in small laminæ. It is soluble in water, and has an alkaline reaction. It is precipitated by the general reagents used in testing for such bodies. Analysis of the base itself and of its platinochloride correspond with the formula $C_3H_5N_3O$; and the various reactions of this ptomaïne, and the products of its decomposition, prove that it has the constitution of glycocyamidine:



This ptomaïne is very poisonous, and, when administered to a cat, it produced high fever (40° C.), and death within thirty-six hours.

---

[1] Dr. A. B. Griffiths, *Comptes Rendus de l'Académie des Sciences* (Paris), tome cxiv. p. 496; *Bulletin de la Société Chimique de Paris*, 1892, 3ᵉ série, tome vii. p. 250.

(b) From Whooping-Cough.—The ptomaïne which occurs in this highly infectious disease is a white crystalline substance. It has the formula $C_5H_{19}NO_2$.

(c) From Erysipelas.—The poisonous ptomaïne extracted from urine in cases of erysipelas has the formula $C_{11}H_{13}NO_3$.

These three ptomaïnes are not present in normal urines, consequently they are produced in the system during the diseases.

IX. BRIEGER'S METHOD FOR ISOLATING PTOMAÏNES.

This process has been already alluded to, but it should be stated that after the oxy-aromatic acids have been driven off, the H_2SO_4 is precipitated by baryta, and the precipitate removed by filtration. The excess of baryta is precipitated by CO_2, and the $BaCO_3$ also removed by filtration. The filtrate is then heated on a water-bath, cooled, and precipitated with $HgCl_2$. The precipitate is washed and decomposed by H_2S; the HgS is filtered off, and the filtrate concentrated. The mineral salts, etc., crystallise out first and are rejected, then the dried residue is treated with absolute alcohol, which, after concentration, deposits the hydrochlorides of the ptomaïnes. These are separated by fractional precipitation with platinic chloride, auric chloride, etc.

'In some of his researches, Brieger[1] has shortened the process by precipitating the putrid fluids, after boiling and filtering directly, with $HgCl_2$, *i.e.* the first precipitation with lead acetate is omitted. As $HgCl_2$ does not precipitate all ptomaïnes, both precipitate and filtrate must be examined.'

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[1] *Die Ptomaïne*, 1885-1886 (3 parts).

X. MICROBES OF SOIL, WATER, AND AIR.

Concerning our previous remarks on the microbes of the soil, it may be added that Reimers[1] has recently ascertained the number of microbes in soil at various depths. For instance—

A sample of soil at surface contained	2,564,800	microbes per cc.
" " 2 yards below surface contained	23,100	" "
" " 3½ " " " "	6,170	" "
" " 4½ " " " "	1,580	" "
" " 6 " " " "	0	" "

It has been already stated that *Bacillus tetani* has been found in soil; and Macé[2] has recently found *B. typhosus* in various samples of soil. *B. tuberculosis* and *B. coli communis* have also been found in soil. On the other hand, De Giaxa[3] has shown that soil is a bad medium for the preservation of *B. choleræ Asiaticæ*, this being due to the large number of saprophytic species present, whose struggle for existence interferes with the vitality of the cholera microbe. In fact, this is an important example of the survival of the fittest, for De Giaxa has proved that soil *per se* has no detrimental action on the cholera microbe.

If soil is a bad medium for the preservation of some forms, water (especially when polluted) is a better medium for others. At the recent Congrès d'Hygiène Ouvrière[4] M. Gautier exhibited illustrations of the typhoid, carbuncle, cholera, and diphtheritic microbes, with many others, in Seine water;[5] and the distinguished chemist said to householders and others,

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[1] *Zeitschrift für Hygiene*, vol. vii.

[2] *Comptes Rendus*, vol. cvi. p. 1564; and his *Traité Pratique de Bactériologie* (1891), p. 717.

[3] *Annales de Micrographie* (1890) vol. ii.

[4] Held in Paris during April 1892.

[5] Paris drinking-water.

'Do not fear these foes. If they hurt you, it is because you drink unfiltered water and eat ill-baked bread. Filter your water or boil it, and if your bread seems ill-baked, toast it well or let it stay some time in a hot oven.'

If householders, corporations, and others would attend a little more to the ordinary rules of health—such as filtering water, boiling milk, destroying unsound food, removing refuse, isolating infectious persons, disinfecting articles of an infectious nature, etc.—there would be a considerable decrease in the number of infectious cases, especially during the time of epidemic diseases. In fact, these rules would go a long way towards the prevention of such diseases.

There is no doubt that many of the epidemics of cholera and other infectious diseases have been largely due to bad or imperfect sanitation. In densely-populated centres it is imperative that the most perfect rules of sanitation should be practised by corporations, sanitary authorities, householders, and others. One cannot help but believe that the visitations of epidemic diseases in the past have been blessings in disguise, because they have taught us that cleanliness in all things (in person, food, drink, home, and city) tends directly to prevent and combat the attacks of such diseases as cholera, typhoid fever, scarlatina, etc. In past times town authorities and householders did not heed the voice of the cholera fiend, as is sung in Mackay's lyric, 'The Cholera Chant'—

'They will not hear the warning voice.
The cholera comes,—rejoice! rejoice!
He shall be lord of the swarming town!
And mow them down, and mow them down!'

Although there is still room for improvement in sanitary matters, yet no one can be blind to the fact that, in every direction, sanitation has made rapid progress in Great Britain.[1]

If, by observing such rules as those specified, we can keep in check the obnoxious microbes in water and food, it is not such an easy matter to deal with those present in the atmosphere.[2] But even aëreal microbes (those spirits of the air) may be, to a large extent, kept in check by the use of disinfectants.

XI. STATISTICS CONCERNING ZYMOTIC DISEASES.

The *Quarterly Report of the Registrar-General*, relating to the deaths in England and Wales from zymotic diseases, gives the following figures:—

5202	deaths	from	whooping-cough.
2769	,,	,,	measles.
1306	,,	,,	diphtheria.
1078	,,	,,	scarlatina.
1361	,,	,,	diarrhœa.
890	,,	,,	'fever' (chiefly enteric).
76	,,	,,	small-pox.

The above figures give a total of 12,682 deaths from zymotic diseases during the first three months of 1892.

---

[1] For those interested in sanitary matters, the author recommends Dr. A. C. Maybury's excellent *Epitome of the Public Health Act*, 1891 (H. Kimpton, 82 Holborn, London).

[2] As microbes are always present in air, soil, and water, it may well be asked, 'Where do they come from?' We know not where; perhaps from the djinnistan of the Persians.

LIST OF FIRMS WHERE BACTERIOLGICAL APPARATUS, ETC., CAN BE OBTAINED.

Microscopes, etc.

C. Zeiss, Jena, Germany; or Zeiss's agent, C. Baker, 244 High Holborn, London.

Incubators, Sterilisers, etc.

F. E. Becker & Co., 33 Hatton Wall, London; R. Muencke, 58 Luisenstrasse, Berlin; R. Kanthack, Imperial Mansions, Oxford Street, London.

Chemical Apparatus and Chemicals.

J. Orme & Co., 65 Barbican, London.

Staining Solutions, etc.

F. E. Becker & Co., 33 Hatton Wall, London; R. Kanthack, Imperial Mansions, Oxford Street, London.

Agar-agar and Gelatine.

Christy & Co., 25 Lime Street, London; J. F. Shew & Co., 89 Newman Street, Oxford Street, London; R. Kanthack, Imperial Mansions, Oxford Street, London.

Microtomes.

Cambridge Scientific Instrument Co., Cambridge; R. Kanthack, Imperial Mansions, Oxford Street, London.

Dissecting Knives, etc.

C. Baker, 244 High Holborn, London.

Mr. Kanthack furnishes estimates of the requirements of a completely fitted bacteriological laboratory.

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